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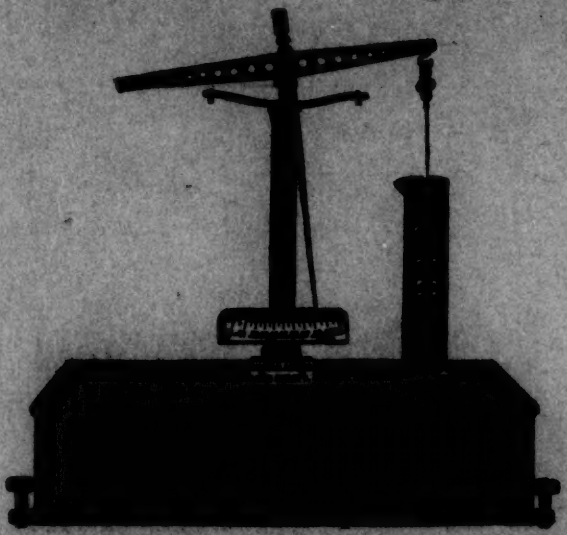

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LIGHT PRODUCTION AT LOW TEMPERATURES BY CATALYSIS WITH METAL AND METALLIC OXIDE HYDROSOLS.

By B. C. GOSS.

(*From the Department of Chemistry, Princeton University, New Jersey.*)

(Received for publication, June 1, 1917.)

INTRODUCTION.

Reactions which are capable of producing light at low temperatures are of considerable interest because of the probable similarity to the processes occurring in the luminous organisms. Lophin, esculin, several essential oils, and certain alcohols and aldehydes have been known to luminesce in the presence of sodium and potassium hydroxide, all of them requiring, however, a higher concentration of alkali than is compatible with life (Radziszewski, 1887, 1880; Dubois, 1901; Trautz, 1905).

More recently, lophin and esculin have been found by Ville and Derrien (1913) and by Dubois (1913) to luminesce with blood and hydrogen peroxide, the hemoglobin acting as an oxidase. Harvey (1916) has described a reaction which shows the general character of these luminescent reactions. He found that the "oxidation of a mixture of pyrogallol and hydrogen peroxide by vegetable oxidases occurs with the production of light." In this reaction the mixture of hydrogen peroxide and pyrogallol corresponds to the oxidizable material occurring in the luminous organism, the vegetable oxidase to the oxidizing enzyme, both of which must be present in order that oxidation may proceed with production of light. The oxidases used by Harvey were usually potato or turnip juice or a 1 per cent extract of ox blood. He found that perceptible light could be produced with concentrations of pyrogallol as low as 0.000031 M, becoming brighter with increased concentration up to 0.00012 M, above which there was little change. The light increased in brightness with increased concentration of oxidase and also with a rise in temperature, a faint light being produced at 0°, a bright light at 10°C. The oxidase was destroyed in the course of the reaction and was therefore not a real catalyst but one factor in what Bayliss (1915) calls a "coupled reaction." Other oxidases were tried and found to give light, among which were horseradish and sweet potato juice and the extracts of several invertebrates. Certain inorganic substances, such as

potassium ferrocyanide, ferric chloride, and potassium permanganate, could take the place of the vegetable oxidase.

The suggestion was made by Bach (1897) and later by Kastle and Loevenhart (1901) that the action of oxidases depends on the formation by these bodies of unstable peroxide-like substances which can give up their oxygen in turn to an oxidizable substance present in the cell. Bach and Chodat (1903) separated an oxidase into two constituents, one of a peroxide nature which they called "oxygenase," another which could excite the oxygenase to activity, called a "peroxidase." While the origin and exact nature of the oxygenase are still somewhat doubtful, it is generally accepted that biological oxidations are brought about by oxidases which consist of two substances, oxygenases and peroxidases.

In many reactions the behavior of plant peroxidases has been found to be closely imitated by platinum and palladium sols. The effect of these metals, in the colloidal state, on the decomposition of hydrogen peroxide (Paal, 1907), the union of hydrogen and oxygen (Paal, 1916, *a*), and the oxidation of carbon monoxide to carbon dioxide (Paal, 1916, *b*) has been studied, as well as the transfer of oxygen from hydrogen peroxide to gum guaiac by Reed (1916, *a*) who observed that different samples of colloidal platinum and silver behaved differently toward guaiac, in some cases bringing about oxidation directly, in others only in the presence of hydrogen peroxide.

These facts suggested the possibility that certain of the metal and metallic oxide sols might be able to take the place of the vegetable oxidases in the so called luminescent reactions. Experiments have therefore been made to determine the effect of various colloids on the oxidation of pyrogallol, the results of which are presented in this preliminary paper.

EXPERIMENTAL.

The reaction used in this study of luminescence was the one described by Harvey (1916) in which a half and half mixture of 0.01 M pyrogallol and 3 per cent hydrogen peroxide (McKesson and Robbins, commercial, neutralized by the addition of 0.1 N sodium hydroxide) was added to an equal volume of the vegetable oxidase solution. 1 cc. each of the pyrogallol and hydrogen peroxide were placed in a test-tube together and a series of such tubes arranged in a rack directly in front of the observer. Directly in front of these tubes a corresponding series was placed containing 2 cc. of the colloidal sol whose peroxidase activity was to be tested. All observations were made after a period of

TABLE I.

Catalysis of Light Production by Colloidal Metals and Oxides.

Sol.	Method of preparation.	Character of light produced.*
Silver.	Suspension of freshly precipitated silver oxide in water and reduced by a stream of hydrogen gas at 70°C.	−**
"	" in 0.1 per cent dextrin.	−
"	" " 0.1 " " potassium stearate.	−
"	" in 0.1 per cent dextrin + 0.001 N NaOH.	+
"	Paal's, protected, sodium protalbate (Paal, 1904).	++
"	Bredig arc dispersion, 4 amperes, in conductivity water.	++
"	" " in 0.001 N NaOH.	+++
Platinum.	" " dispersion in conductivity water.	+++
"	" " " in 0.001 N NaOH.	+++
Palladium.	" " " "	+++
Gold.	" " " "	+
Copper, copper oxide.	" " " "	−
Ferric oxide.	Peptonization.	−
Manganous oxide.	From freshly precipitated hydroxide by hydrogen at 70°C.	+
" "	Paal's, protected, sodium protalbate.	−
Silver "	" " " "	−
Copper "	" " " "	−
Nickel "	" " " "	−
Chromium "	" " " "	+
Cobalt "	" " " "	+++

* The intensity of the light produced has been described as faint, fair, or bright; faint light has been indicated by +, fair light by ++, and bright light by +++. If no light was visible it has been indicated as −.

** Negative results in the case of the silver sols prepared by reduction of the freshly precipitated oxide were probably due in part to the fact that only very dilute sols could be obtained in this way. The addition of dextrin, potassium stearate, sodium glycocholate, etc., resulted in the formation of a more concentrated sol, presumably because of the lowering of surface tension.

15 minutes in total darkness. In these reactions the maximum light was usually developed within ½ minute after the time of mixing and was quite transient, having disappeared within 2 or 3 minutes.

Observations were first made on a variety of sols, prepared by different methods, in order to determine which metals and oxides were effective in the catalysis of this reaction. The results are shown in Table I.

In order to compare the activity of platinum, palladium, silver, and gold in the transfer of oxygen, sols of these metals were prepared in 0.001 N NaOH by the Bredig arc dispersion method. After letting stand for a day and decanting the solution away from the large particles which had settled out, the gross concentration was determined by evaporating a known volume of the sol and weighing the residue. By successively diluting these solutions with distilled water, the minimum concentration was found which would just produce visible light.

The effect of potassium stearate in increasing the intensity of the light was unexpected. Colloidal sols of gold, silver,

TABLE II.

*Minimum Concentration of Colloidal Metal Necessary to Produce Visible Light.**

Silver.		Platinum.		Palladium.		Gold.	
Concentration.	Intensity of light.	Concentration.	Intensity of light.	Concentration.	Intensity of light.	Concentration.	Intensity of light.
gm./cc.		*gm./cc.*		*gm./cc.*		*gm./cc.*	
0.000200	++	0.000120	+++	0.0000960	+++	0.00024	+
0.000100	+	0.000060	+++	0.0000480	+++	0.00012	+
0.000050	+	0.000030	+++	0.0000240	++	0.00006	−
0.000025	−	0.000015	++	0.0000120	+		
0.000012	−	0.000008	+	0.0000060	+		
		0.000004	+	0.0000030	−		
		0.000002	−				

* The values obtained here would doubtless depend to a considerable extent upon the size of the particles. The figure obtained for the minimum concentration of gold required to produce visible light would, therefore, be farther from that of platinum than appears from the above table since the particles in the red gold sol used were probably in the range of 20 to 30 $\mu\mu$ in diameter, while those of the platinum were larger, 40 to 50 $\mu\mu$.

platinum, and palladium were taken of the concentration which would just produce visible light and the effect was compared with that produced by the same concentration of metal in 0.5 per cent potassium stearate. In each case the light was markedly increased. Potassium stearate alone gave no light. Isocapillary solutions (stalagmometer method) of potassium stearate, sodium stearate, and potassium oleate, containing 0.000017 gm. per cc. of palladium were tried and it was found that potassium stearate increased the light greatly, sodium stearate increased it slightly, while potassium oleate cut off all light. The same relative effect was observed at 80°C., above the melting point of the stearic acid formed by hydrolysis.

TABLE III.

Effect of Protective Colloids in Inhibiting the Production of Light.

Dispersion medium.		Light produced.	
		Platinum sol, Bredig 0.000008 gm./cc.	Silver sol, Bredig 0.0002 gm./cc.
Water		+	++
Gelatin	0.5 per cent	−	−
Egg albumin	0.5 " "	−	−
Potassium oleate	0.5 " "	−	−
Saponin	0.5 " "	−	−
Potassium stearate	0.5 " "	+++	+++
Tannic acid	0.5 " "	−	−
Sodium glycocholate	0.5 " "	−	−
Gum arabic	0.5 " "	−	−
Agar	0.5 " "	−	+
Dextrin	0.5 " "	−	+
Gum tragacanth	0.5 " "	−	+

The addition of alcohols increased the light in general, the higher alcohols producing the greater effect in equimolar concentrations. Of those tried, capryl > amyl > butyl > propyl > ethyl > methyl. Ethyl and methyl gave results differing little from that in pure water. This action of the higher alcohols cannot be explained on the basis of lowering of surface tension alone, since even when isocapillary solutions were used (Lillie, 1916) the order of effect was amyl > propyl > methyl. Further investigation will be necessary before many of the observed phenomena can be explained.

DISCUSSION.

It has been observed by Harvey that the intensity of the light produced does not run parallel to the rate of evolution of oxygen. This has been confirmed by my experiments, since in some cases a violent bubbling off of oxygen occurred when little or no luminescence was present. It is evident, too, that the effect of the colloidal metal was not due entirely to the large surface exposed and the consequent adsorption of oxygen, since widely different results were obtained with metal sols of approximately the same degrees of dispersion. The colloidal metals which were most active in transferring oxygen from the hydrogen peroxide to the pyrogallol were those which are able to form unstable compounds with oxygen. Some interesting experiments along this line were described by Reed (1916, *a*), showing that when finely divided platinum (platinum black, incorrectly called "colloidal platinum") on an electrode is charged with oxygen by making it the anode, it can bring about oxidations directly. Platinum charged with hydrogen cannot oxidize directly, yet both are able to decompose hydrogen peroxide. The author (Reed, 1916, *b*) draws the conclusion that "although substances which act as oxidases or peroxidases usually decompose hydrogen peroxide, yet in the case of colloidal platinum they are quite independent."

It must be pointed out, however, that in the decomposition of hydrogen peroxide by colloidal platinum it is only the rate of a reaction already occurring which is changed, and that a sample of platinum black cannot be placed in contact with a solution of hydrogen peroxide without immediately taking up oxygen. I have observed, for example, that if finely divided platinum which showed no direct oxidase action, *i.e.*, was unable to blue gum guaiac, was placed in hydrogen peroxide for a few minutes and then removed and washed with four changes of distilled water, it did show direct oxidizing action by blueing guaiac solution. This was not due to an adsorption of hydrogen peroxide as such, as has been suggested by some authors, since the same result was obtained with colloidal platinum sols by bubbling air through them or, more slowly, by letting them stand in contact with air. Platinum, palladium, and silver sols, as prepared by the arc dispersion method in water, showed this presence of oxygen by blue-

ing guaiac directly without the presence of hydrogen peroxide. If a stream of hydrogen was bubbled through the sol for a few hours, the colloidal metal lost the power to oxidize guaiac directly but produced oxidation by hydrogen peroxide, or, if let stand in air for only a few minutes, the colloid took up oxygen and regained its oxygenase activity.

Bose (1900), after summarizing the work done previous to that time, concluded from the experimental evidence that the combination of hydrogen and oxygen with platinum, palladium, and gold was not due to the formation of compounds, but to solid solution, together with a certain amount of adsorption. He noted that, while the metal had a greatly differing capacity for hydrogen and oxygen, the total of absorbed and adsorbed gas became greater with subdivision of the metal, and that the difference between the capacity for hydrogen and oxygen became less. Later investigations have supported this view regarding the condition of the hydrogen, but have indicated the formation of a series of oxides of platinum and palladium. Lorenz (1906) noticed that the E.M.F. of cells with platinum electrodes differed widely among themselves and suggested that the E.M.F. of the oxygen electrode was determined by the formation of an oxide. He found that the oxides of cadmium, copper, silver, and iron gave an E.M.F. which was identical with that of the metal when measured under the same conditions. Wöhler (1905) studied the oxides of palladium and their behavior toward reducing agents, and found that palladium dioxide, although a strongly exothermal compound, was a better oxidizing agent, toward many substances, than molecular oxygen. He suggested that this behavior was due to the easy splitting off of atomic oxygen. Hydrogen peroxide reduces palladium dioxide much more readily than platinum dioxide, but the reverse order is true of the monoxides, platinum monoxide being easily reduced by organic acids or by hydrogen peroxide. Palladium metal catalyzed the decomposition of hydrogen peroxide more vigorously than palladium dioxide, which, in turn, decomposed the peroxide more rapidly than the monoxide. The authors concluded that the decomposition of the hydrogen peroxide was brought about, not by palladium monoxide or dioxide necessarily, but perhaps by an intermediate oxide.

It appears from these experiments that, in the case of the col-

loidal metals, catalase activity and the ability to transfer oxygen, *i.e.*, oxidase activity, are not at all independent but that both are dependent upon the ability of the metal to combine with oxygen to form an unstable complex which is a better oxidizing agent than either hydrogen peroxide or molecular oxygen. This combination is probably due to adsorption of oxygen on the great surface exposed, together with the formation of oxides as the result of this adsorption, the oxide being soluble in the metal. The oxidation of the pyrogallol in this reaction mixture was brought about, therefore, by both the adsorbed and combined oxygen, the proportion of adsorbed oxygen becoming greater with decreased size of particles. The colloidal metal is continually supplied with oxygen by the hydrogen peroxide, whose normal decomposition according to the equation

$$H_2O_2 \rightleftarrows \overset{+}{H} + \overset{-}{HO_2} \rightleftarrows HO + \overset{-}{O}$$

is accelerated by the removal of oxygen atoms. The metal-oxygen complex furnishes "active" oxygen by dissociating in contact with the pyrogallol.

SUMMARY.

White light was obtained by the oxidation of pyrogallol with hydrogen peroxide in the presence of certain colloidal metal and metallic oxide sols, closely resembling that produced by luminous organisms.

The action of the colloidal metal was similar to that of the vegetable oxidase, except that in the former case the catalyzer was not destroyed.

Visible light was produced by concentrations of colloidal platinum as low as one part in 250,000.

A platinum sol containing 0.0002 gm. per cc. produced a fair light at $-5°$C. and a bright light at 0°C.

This catalysis was not due entirely to the high degree of dispersion of the metal or oxide and the consequent large specific surface, but was also, in part, dependent upon the ability of the metal to form unstable compounds with oxygen.

The production of light was inhibited, in general, by the presence of protective colloids such as gelatin and egg albumin.

Potassium stearate markedly increased the intensity of the light produced, the concentrations of the other substances remaining constant. A similar effect was observed in the presence of the higher alcohols such as capryl and amyl, suggesting the influence of surface tension.

The author wishes to express his appreciation of the assistance given by Drs. E. N. Harvey and Alan W. C. Menzies in this investigation. A closely related paper by Harvey, who has noticed independently that oxidation with light production may be brought about by colloidal silver and platinum, appears on page 311.

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BLOOD FAT IN DOMESTIC FOWLS IN RELATION TO EGG PRODUCTION.*

By D. E. WARNER and H. D. EDMOND.

(*From the Poultry and Chemical Laboratories of the Storrs Agricultural Experiment Station, Connecticut.*)

(Received for publication, June 2, 1917.)

The natural color of the body fat of most hens and the fat in egg yolk is yellow. This color is due to a pigment of the xanthophyll group with a very little "carotin-like pigment," as shown by Willstätter and Escher in their isolation of it in crystalline form from eggs,[1] and identical with plant xanthophyll as shown by Palmer.[2] It was the disappearance of this color from the external parts of the hen's body that led the writers to make the present investigation. There is a correlation between egg laying activity and yellow pigment in the domestic fowl. Blakeslee and Warner[3] have shown that where the yellow in the ear lobes did not exceed 20 per cent in a given group of hens the per cent of birds laying at that time was high; but with birds having a higher amount of yellow there was a decline in per cent laying. The conclusion drawn by Blakeslee and Warner[4] was that "the laying removed the yellow pigment from the body for the production of eggs more rapidly than it could be replaced by the normal metabolism." With this fact in mind the writers believed that if the yellow pigment which was present in the hen's body previous to its laying was transferred to the egg yolk, in like manner the body fat which contains the yellow pigment would be taken from the body by the blood to assist in the build-

* By fat in this paper we mean the total ether extract composed chiefly of fats and cholesterol.

[1] Willstätter, R., and Escher, H. H., *Z. physiol. Chem.*, 1911–12, lxxvi, 214.

[2] Palmer, L. S., *J. Biol. Chem.*, 1915, xxiii, 261.

[3] Blakeslee, A. F., and Warner, D. E., *Am. Naturalist*, 1915, xlix, 360.

[4] Blakeslee and Warner, *Science*, 1915, xli, 432.

ing up of the egg yolk. If this is the case a hen which is laying heavily should have blood much richer in fat than a hen that is not laying.

The object of this paper is to show the relationship of blood fat in fowls to (1) egg production, (2) presence of food in the alimentary tract, (3) color of legs, etc., and (4) sex.

Warner[5] has shown that the average amount of fat found in the high producing hens and hens that were laying was 1.426 per cent; in the low producers it was 0.886 per cent. This, however, was preliminary work and involved only ten hens.

These results were considered worthy of a further study. In October, 1916, eighty-two hens and twelve cockerels belonging to the Storrs Agricultural Experiment Station were used for the following work. All of the hens used for this work were White Leghorns that had just completed their 1st or 3rd year of laying, and their trap nest records were at hand. Among this group of females were found birds having high, mediocre, and low fecundity.

EXPERIMENTAL.

In considering various procedures for the determination of fat in blood the methods of Kumagawa and Suto,[6] Rosenthal and Trowbridge,[7] and Bloor[8] seemed to require too much time and manipulation without compensating advantages. A modified Soxhlet method for fat, similar to the one used by Gettler and Baker,[9] was used for this work.

About 5 gm. of blood were drawn from the basilar vein into a small test-tube. This blood was poured into a thin-walled lead dish of known weight, containing a mat of asbestos fiber, and the whole was quickly weighed. The dish and contents were then dried over phosphorus pentoxide *in vacuo* for 12 hours at 50–60°C., and kept over phosphorus pentoxide until extraction was

[5] Paper read before the Ohio meeting of the American Association of Instructors and Investigators in Poultry Husbandry, August, 1916; *J. Am. Assn. Instructors and Investigators in Poultry Husbandry*, 1916, iii, No. 1, p. 4.

[6] Kumagawa, M., and Suto, K., *Biochem. Z.*, 1908, viii, 212.

[7] Rosenthal, H., and Trowbridge, P. F., *J. Biol. Chem.*, 1915, xx, 711.

[8] Bloor, W. R., *J. Biol. Chem.*, 1914, xvii, 377.

[9] Gettler, A. O., and Baker, W., *J. Biol. Chem.*, 1916, xxv, 218.

made. After an 18 hour extraction the ether was evaporated from the extracted fat and the extraction flask dried over phosphorus pentoxide *in vacuo* to constant weight at 50–60°C. By the use of this process the extracted material was found to be completely free from blood pigments and only possessed the yellow color of fat when it was present in the larger amounts; otherwise the extract was colorless. The extraction as described above gave fat, cholesterol, and fatty acids, and probably also traces of other lipoids.[9] For the discussion in this paper the entire extracted material is called fat. The cholesterol was determined in the blood of a few laying and non-laying hens and also a few roosters, as shown in Table X. The average amount of cholesterol for these laying and non-laying hens was more uniform than the fat for these same groups, and no particular correlation could be traced. It is planned, however, to make a further study of the cholesterol and fat in the domestic fowl.

The method used for the determination of cholesterol was that used by Gettler and Baker, as directed by Autenrieth and Funk[10] and also by Bloor.[11] As outlined by the above method, the fatty extract was taken up with 5 cc. of chloroform to which were added 2 cc. of acetic anhydride and 1 cc. of sulfuric acid, and made up to 10 cc. with chloroform. The solution was then kept in the dark for 15 minutes and compared in a Duboscq colorimeter with a similarly treated standard solution of cholesterol in chloroform.

Table I shows that the total per cent of fat found in the blood of seventy hens was 28.467 per cent or a mean of 0.407. The per cent of fat found in the blood of hens is variable and the average per cent of fat found in this group of Leghorn females is much lower than that reported by Ingles.[12] The per cent of fat varies from 0.083 to 1.953, which is quite striking. The variation is due largely to the fact that the birds that had a high per cent of fat in their blood were all laying at the time the samples of blood were taken, whereas most of the others were not laying.

[10] Autenrieth, W., and Funk, A., *Münch. med. Woch.*, 1913, lx, 1243.

[11] Bloor, *J. Biol. Chem.*, 1916, xxiv, 227.

[12] Ingles, H., Manual of Agricultural Chemistry, London, 1908, 259. The amount of non-protein reported by Ingles would, however, include fat, mineral matter, and possibly some organic substances.

TABLE I.

Fat in the Blood of Seventy Hens That Had About Completed Their 1st Year of Laying.

No.	Fat.	No.	Fat.	No.	Fat.
	per cent		*per cent*		*per cent*
15B– 9	0.083	15B–52	0.180	15B–32	0.258
15	0.085	46	0.181	5L–67	0.272
13	0.088	5L–71	0.183	5L–62	0.300
54	0.114	68	0.187	15B–97	0.307
16	0.123	15B–55	0.187	5L–60	0.312
5L–54	0.123	58	0.188	15B–11	0.406
15B–56	0.124	20	0.189	5L–51	0.437
18	0.125	5L–64	0.191	15B–23	0.448
3	0.131	77	0.195	64	0.448
60	0.131	15B–80	0.198	5L–63	0.526
74	0.133	31	0.202	15B–24	0.541
49	0.138	36	0.202	39	0.625
5L–53	0.144	37	0.204	5L–70	0.735
15B–51	0.154	66	0.204	57	1.255
19	0.161	34	0.205	15B– 5	1.315
5L–66	0.166	25	0.223	89	1.319
15B– 1	0.167	62	0.228	5L–65	1.388
83	0.167	98	0.228	15B–90	1.641
5L–52	0.167	104	0.230	5L–59	1.779
15B–96	0.169	82	0.231	15B–57	1.864
5L–55	0.175	77	0.241	43	1.915
15B–38	0.178	91	0.242	12	1.953
99	0.179	5L–69	0.246	Total.	28.467
105	0.180	15B–28	0.253	Mean.	0.407

Table II shows the per cent of fat found in the blood of the 3 year old hens. These data show that the blood was very low in fat, giving an average of only 0.171 per cent. There is a marked difference between the amount of fat found in this group and that found in the blood of 1 year old hens. None of the 3 year old birds had laid, on the average, for 60 days at the time the blood samples were taken. This fact we believe warrants the conclusion that a low fat content signifies that the hen is not laying.

Of the eighty-two hens there were, then, only seventeen that were laying when the blood samples were taken; the average number of eggs from these hens was 163.7, showing a much higher average egg production than for the birds that were not laying

TABLE II.

Fat in the Blood of Twelve Hens That Had About Completed Their 3rd Year of Laying, and Days since Their Last Egg Was Laid.

No.	Fat.	Days since last egg was laid.
	per cent	
107	0.066	56
425	0.100	46
76	0.126	73
382	0.157	36
43 RR	0.161	92
401	0.166	51
430	0.168	63
386	0.176	35
102	0.194	61
404	0.208	66
116	0.228	79
110	0.297	62
Total	2.047	720
Mean	0.171	60

TABLE III.

Laying Hens.

No.	Yearly total.	Fat.
		per cent
69	132	0.246
15B–32	158	0.358
11	168	0.406
23	135	0.448
5L–63	147	0.526
15B–39	138	0.625
5L–70	176	0.735
57	144	1.235
15B– 5	227	1.315
89	186	1.319
5L–65	142	1.388
15B–90	211	1.641
5L–59	107	1.779
15B–57	172	1.864
43	179	1.915
12	192	1.953
Total	2,615	17.753
Mean	162.8	1.109

TABLE IV.

Non-Laying Hens.

No.	Yearly total.	Fat.	Days since last egg was laid.
		per cent	
15B– 9	178	0.083	61
15	120	0.085	66
13	202	0.088	28
54	113	0.114	110
16	139	0.123	66
5L–54	148	0.123	36
15B–56	124	0.124	20
18	186	0.125	9
3	168	0.131	78
60	153	0.131	22
74	165	0.133	28
49	220	0.138	10
5L–53	144	0.144	65
15B–51	154	0.154	6
19	156	0.161	61
5L–66	134	0.166	55
15B– 1	166	0.167	60
83	102	0.167	58
5L–52	147	0.167	23
15B–96	146	0.169	17
5L–55	134	0.175	22
15B–38	156	0.178	36
99	189	0.179	6
105	101	0.180	35
52	10	0.180	212
46	110	0.181	83
5L–71	140	0.183	46
68	133	0.187	88
15B–55	150	0.187	22
58	198	0.188	13
20	141	0.189	26
5L–64	170	0.191	6
77	101	0.196	60
15B–80	149	0.198	8
31	113	0.202	51
36	150	0.202	39
37	167	0.204	33
66	72	0.204	82
34	171	0.205	24
25	134	0.223	38

TABLE IV—*Concluded.*

No.	Yearly total.	Fat.	Days since last egg was laid.
		per cent	
62	96	0.228	95
98	117	0.228	55
104	138	0.230	53
82	113	0.231	54
77	166	0.241	10
91	171	0.242	22
28	132	0.253	51
5L–67	91	0.272	55
5L–62	146	0.300	62
15B–97	152	0.307	43
5L–60	149	0.312	37
51	139	0.437	50
15B–64	0	0.448	365
24	147	0.541	32
Mean	139.09	0.199	51.7

when the blood samples were taken. The average per cent of fat found in the blood was much higher for birds that were laying than for the group of birds that were not laying (Tables III and IV). This is very striking and quite agrees with the averages reported earlier.[5] One-half of the birds had over 1 per cent of fat in their blood, as shown in Table III.

Table IV shows that the per cent of fat in the blood of non-producers was very much lower than the per cent of fat in the blood of laying birds. Every bird in this group had stopped laying from 6 to 365 days previous to the time the blood samples were taken. The average egg production for this group of birds was 139.1, which is a much lower average than for the birds that were laying when the samples were taken. Not only this, but the average per cent of fat was much lower, while the average days since they had produced any eggs was 51.7. Only one individual in this group of birds had over 0.5 per cent of fat in the blood while others gave a reading as low as 0.083 per cent.

Table V shows that if all the birds having over 1 per cent of fat in their blood are grouped together the average egg production is found to be 173.4, or an average of over 55 eggs more than that of the birds that had yellow beaks, legs, and vents,

TABLE V.

Data for Hens with Over 1 per Cent of Fat in the Blood, and with Pale Beaks, Legs, and Vents.

No.	Yearly total.	Fat.	Days since last egg was laid.
		per cent	
5L–57	144	1.235	0
15B– 5	227	1.315	0
89	186	1.319	0
5L–65	142	1.388	0
15B–90	211	1.641	0
5L–59	108	1.779	0
57	172	1.864	0
43	179	1.915	0
12	192	1.953	0
Total.....	1,561	14.409	0
Mean.....	173.4	1.601	0

and a low fat content. Further, this group of birds were all laying when the records were taken, and higher annual egg production would of course be expected from birds that had continued to lay until October, the time the blood samples were taken.

As a rule, if a bird has been laying for some time the legs, beak, and vent undergo a physiological change, and where they continue to lay, these parts will become decidedly pale in color. Table VI shows that what is true of the external parts of the body is quite true of the blood. That is, the blood of birds having external parts that have bleached have on the average a higher per cent of fat in their blood. The fat content of the blood is correlated with egg laying activity, and there is also a slight correlation between the amount of fat in the blood and high yearly production. The amount of fat is also correlated with the fading of the external parts. There is an average of 0.816 per cent of fat in the blood of hens having pale beaks, legs, and vents. When this is compared with the data for the group of hens having these parts yellow there is found to be only an average of 0.196 per cent of fat (Tables VI and VII).

Table VII shows the reverse of Table VI in the average yearly total per hen, average per cent of fat, and average number of days since the last egg was laid. The average production was only 117.9 for the birds having yellow legs, beaks, and vents,

TABLE VI.

Data for Hens with Pale Beaks, Legs, and Vents.

No.	Yearly total.	Fat.	Days since last egg was laid.
		per cent	
15B–60	153	0.131	22
51	154	0.154	6
99	189	0.179	6
105	101	0.180	35
5L–64	170	0.191	6
15B–77	166	0.241	10
32	158	0.258	0
11	168	0.406	0
23	135	0.448	0
5L–63	147	0.526	0
70	176	0.735	0
15B–5	227	1.315	0
89	186	1.319	0
5L–57	144	1.235	0
15B–90	211	1.641	0
57	172	1.864	0
43	179	1.915	0
12	192	1.953	0
Total.....	3,028	14.691	85
Mean.....	168.20	0.816	4.72

whereas the average production for the birds that were pale in those parts was 168.2. The per cent of fat found in this group of birds was only 0.196, whereas in the group of birds having pale parts it was 0.816. The average number of days since the last eggs were laid for the group of birds having pale parts was 4.722, while for the group of birds in Table VII it was 75.21. This would indicate that if a bird had yellow legs, yellow beak, and a yellow vent, the per cent of fat in the blood would be low.

It has been reported by Matthews that in man and animals the blood is much richer in fat after they have been eating than it is after fasting. This is not true with hens, as shown from Table VIII; the average per cent of fat for the birds that had been without food is a little higher than those that had not. The difference is not great, however, 0.009 per cent, but would indicate that fasting for 16 hours has no effect upon the amount of fat in the blood.

TABLE VII.

Data for Hens with Yellow Beaks, Legs, and Vents.

No.	Yearly total.	Fat.	Days since last egg was laid.
		per cent	
107x	110	0.066	56
15B- 9	178	0.083	61
15	120	0.085	66
425x	120	0.100	46
15B-54	113	0.114	110
16	139	0.123	66
5L-54	148	0.123	36
76x	111	0.126	73
15B- 3	168	0.131	78
5L-53	144	0.144	65
15B-19	156	0.161	61
401x	93	0.166	51
15B- 1	166	0.167	60
430x	107	0.168	63
15B-52	10	0.180	212
46	110	0.181	83
5L-71	140	0.183	46
15B-55	150	0.187	22
102x	113	0.194	61
5L-77	101	0.195	60
15B-31	113	0.202	51
404x	101	0.208	66
116x	83	0.228	79
15B-98	117	0.228	55
62	96	0.228	95
82	113	0.231	54
5L-67	91	0.272	55
110x	128	0.297	62
5L-62	146	0.300	62
60	149	0.312	37
51	139	0.437	50
15B-64	0	0.448	365
Total.....	3,773	6.268	2,407
Mean.....	117.906	0.196	75.21

Table IX shows the per cent of fat found in the blood of male birds. The figures for fat from these few birds indicate that the per cent of fat in the blood of male birds was more constant than in hens. This may be accounted for by the fact that the beaks,

TABLE VIII.

Fat in the Blood of Hens Fasted for 16 Hours and Hens Not Fasted.

Fasted.	Not fasted.
per cent	*per cent*
0.133	0.164
0.154	0.187
0.178	0.188
0.179	0.198
0.202	0.228
0.223	0.231
0.228	0.241
0.230	0.242
0.253	0.258
0.541	0.307
0.625	0.448
1.915	1.864
4.861	4.753
Average.....0.405	0.396

TABLE IX.

Fat in the Blood of Twelve Male Birds.

No.	Fat.
	per cent
108	0.097
104	0.141
99	0.146
96	0.149
7	0.160
98	0.166
65	0.170
0	0.191
B–78	0.194
20	0.200
33	0.246
27	0.249
Total.....	2.109
Mean.....	0.176

legs, and ear lobes of male birds do not change in color and that there is no building of eggs within the body cavity. The reserve fat stored in the body is not used up in the same way that the female uses hers, and consequently there is no cause for the fad-

ing in the parts mentioned above. The average per cent of fat in a male bird is 0.176, or about the same as the per cent found in the blood of 3 year old hens. The blood of Bird 108 was very low in fat. This is suggestive and leads to the investigation whether the male birds that are prepotent can be detected by the fat content of their blood. (By prepotent we mean a male bird which transmits to its female offspring high fecundity.)

TABLE X.

Fat and Cholesterol in the Blood of a Few Laying and a Few Non-Laying Hens, and Also a Few Roosters.

No.	Cholesterol.	Fat.
	Laying hens.	
	per cent	*per cent*
26	0.019	1.915
9	0.047	0.526
32	0.076	0.258
28	0.082	1.864
61	0.110	1.388
76	0.114	0.246
16	0.121	1.953
64	0.149	1.235
34	0.155	0.448
62	0.169	1.779
5	0.214	1.315
Mean	0.114	1.175
	Non-laying hens.	
41	0.023	0.138
35	0.077	0.241
60	0.078	0.180
39	0.081	0.124
68	0.106	0.191
43	0.118	0.448
79	0.121	0.735
Mean	0.086	0.294
	Male birds.	
95	0.069	0.097
96	0.075	0.166
94	0.089	0.170
93	0.110	0.146
Mean	0.086	0.145

SUMMARY.

The present paper is a contribution to the general problem of the relation between the amount of fat and fecundity.

The data comprise the per cent fat valuations and the egg records for eighty-two hens.

The egg records cover a period from November to October inclusive. Blood samples were taken from October 28 to November 3, 1916.

There is little or no correlation between the amount of fat in the hen's blood and her yearly egg yield. On the other hand the blood of a hen laying at the time the sample is taken is much richer in fat than that of a hen that is not laying.

The average per cent of fat found in the seventy hens that had just completed their 1st year of laying was 0.407; for the twelve old hens having just completed their 3rd year of laying the per cent was 0.171; for the twelve 1½ year old male birds it was 0.176.

From the data at hand it seems improbable that high producing hens can be selected merely by sampling their blood and analyzing for fat unless one is careful to take into account whether or not a hen is laying at the time of sampling, and also the season of the year.

Depriving hens of food for 16 hours did not seem to lessen the per cent of fat in their blood.

There exists a close correlation between the color of the beak, legs, and vent and the per cent of fat found in the blood. The birds that had pale legs, pale beaks, and pale ani carried a very high per cent of fat in their blood and also had a high average egg production. The birds that had distinctly yellow legs, beaks, and ani gave a very low average egg production, and the per cent of fat carried in their blood was very low. This would show that birds that were not laying were storing fat in the body cells, and consequently their legs, beaks, and ani would become yellow, the natural color for all American breeds and the Leghorns.

The average per cent of fat found in the blood of the 3 year old hens was much lower than that found in the blood of the 1 year old birds. The per cent of fat found in the blood of male birds was more constant than that of 1 year old hens. There

did not appear to be very much difference between the per cent of fat found in the blood of male birds and that found in the blood of females not laying.

It seems feasible to believe that the principal reason why the blood of laying hens is much richer in fat than that found in birds not laying is that the fat stored in the body tissues is taken up by the blood and carried to the egg yolk.

TRITICO NUCLEIC ACID.

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Recent investigations have definitely indicated that there are two distinct types of nucleic acid, one of which is found in animal tissues and the other in plants. While this distinction rests upon the examination of a large number of nucleic acids of animal origin, our knowledge of plant nucleic acid has been acquired from a study of but two substances; one of these is prepared from yeast (yeast nucleic acid) and the other from the wheat embryo (tritico nucleic acid).

In 1902 Osborne and Harris[1] prepared tritico nucleic acid and showed that like all other nucleic acids it produces phosphoric acid, guanine, and adenine by hydrolysis with dilute mineral acids. But tritico nucleic acid was found to differ from all animal nucleic acids and to resemble yeast nucleic acid by its production of uracil instead of thymine, and pentose instead of levulinic acid. At the time Osborne and Harris' paper appeared cytosine was not known, but Wheeler and Johnson[2] afterwards found cytosine among the hydrolytic products of tritico nucleic acid.

So far as concerns their fundamental groups, therefore, the two plant nucleic acids differ sharply from all animal nucleic acids and are identical with one another.

After Levene and Jacobs[3] had shown that by hydrolysis with ammonia at high temperatures yeast nucleic acid loses its phosphoric acid and produces four nucleosides, it became of great interest to know whether tritico nucleic acid would behave in the

[1] Osborne, T. B., and Harris, I. F., *Z. physiol. Chem.*, 1902, xxxvi, 85.

[2] Wheeler, H. L., and Johnson, T. B., *Am. Chem. J.*, 1903, xxix, 505.

[3] Levene, P. A., and Jacobs, W. A., *Ber. chem. Ges.*, 1910, xliii, 3150.

same manner. Levene and La Forge[4] found this to be the case. They described a very efficient method of preparing tritico nucleic acid and found that the substance yields three of the four nucleosides of yeast nucleic acid (guanosine, adenosine, and cytidine). They made no attempt to find uridine.

Hence, not only in their fundamental groups but in the formation of nucleosides the two plant nucleic acids are seen to be identical so far as experiments have been made, and we shall extend the coincidence to include uridine.

It has very recently been shown in this laboratory[5] that when yeast nucleic acid is hydrolyzed with ammonia at *low temperatures* no phosphoric acid is removed and two nucleotides are formed; *viz.*, guanine mononucleotide and adenine-uracil dinucleotide.

In the following we show that these same nucleotides are produced by tritico nucleic acid. The guanine mononucleotide obtained from tritico nucleic acid does not differ either in its chemical composition or in any of its chemical properties from the substance formerly prepared from yeast nucleic acid. By mild acid hydrolysis it produces guanine but not adenine, and sets free its entire phosphoric acid. By treatment with brucine it forms a crystalline brucine salt which melts at 203°C.

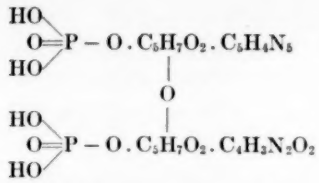
Adenine-uracil dinucleotide prepared from tritico nucleic acid is identical with that prepared from yeast nucleic acid. By mild acid hydrolysis it produces adenine but not guanine, and liberates only half of its phosphoric acid. By hydrolysis with ammonia at high temperatures it produces both adenosine and uridine. It forms with brucine a crystalline brucine salt which has the composition required for the formula $C_{19}H_{25}N_7O_{15}P_2(C_{23}H_{26}N_2O_4)_4.14H_2O$, and melts at 175°C.

The nitrogen percentage of the brucine salt shows that it contains a uracil group, not a cytosine group, and that uracil and uridine are not obtained from tritico nucleic acid as secondary products to cytosine and cytidine. The presence of four brucine radicles in the brucine salt leads to the disaccharide structure of the dinucleotide.[6]

[4] Levene, P. A., and La Forge, F. B., *Ber. chem. Ges.*, 1910, xliii, 3164.

[5] Jones, W., and Read, B. E., *J. Biol. Chem.*, 1917, xxix, 111. Read, B. E., *ibid.*, 1917, xxxi, 47.

[6] For argument see Jones and Read, *J. Biol. Chem.*, 1917, xxix, 113.



Although it is not possible to prove rigidly the identity of two substances the exact coincidence in so many properties of tritico nucleic acid with yeast nucleic acid leaves little room to doubt that they are the same chemical compound.

EXPERIMENTAL.

100 gm. of tritico nucleic acid prepared from the wheat embryo by the method of Levene and La Forge[4] were dissolved in 530 cc. of 2.5 per cent ammonia, and the solution was heated in an autoclave for 1½ hours at 115°. The cooled product was treated with 530 cc. of absolute alcohol when the ammonium salt of guanine mononucleotide was precipitated, leaving the ammonium salt of adenine-uracil dinucleotide in solution. The two substances were separated by filtration, purified, and the two nucleotides finally obtained as white powders by the methods already described in connection with yeast nucleic acid.[5]

Guanine Mononucleotide.—Commercial yeast nucleic acid evidently contains various impurities which contaminate products that are prepared from it. This is not the case with tritico nucleic acid which produces guanine mononucleotide so free from foreign substances that its purification offers little difficulty. The substance which we obtained gave the following analytical data.

I. 0.2634 gm. dried at 100° required 13.65 cc. H_2SO_4 (1 cc. = 0.0037 gm. N).
II. 0.2227 " " " " " 11.50 " " (1 " = 0.0037 " ").
III. 0.4566 " " " " gave 0.1415 gm. $Mg_2P_2O_7$.
IV. 0.4882 " " " " " 0.1525 " "

	N	P
Calculated	19.28	8.54
Found.		
I	19.17	
II	19.11	
III		8.65
IV		8.72

Hydrolysis of Guanine Mononucleotide with Dilute Mineral Acid.—Portions of the guanine mononucleotide were heated with twenty volumes of 5 per cent sulfuric acid and estimations of phosphoric acid and guanine were made with the product.

I. 0.3487 gm. dried at 100° and hydrolyzed for 3 hrs. gave 0.1069 gm. $Mg_2P_2O_7$.
II. 0.3394 " " " 100° " " " 3 " " 0.1032 " " "
III. 0.4924 " " " 100° " " " 1 hr. " 0.1806 " guanine.
IV. 0.7170 " " " 100° " " " 1 " " 0.2677 " "

	P	Guanine
Calculated	8.54	41.6
Found.		
I	8.57	
II	8.49	
III		36.68
IV		37.30

These results show that the substance loses its entire phosphoric acid by mild acid hydrolysis and therefore contains no pyrimidine group.

The final filtrates from magnesium ammonium phosphate gave no gelatinous precipitate of silver-adenine upon treatment with ammoniacal silver solution.

The Brucine Salt of Guanine Mononucleotide.—A solution of 1 gm. of guanine mononucleotide in 5 cc. of hot water was treated with 2.5 gm. of brucine in 5 cc. of hot absolute alcohol. The crystalline dibrucine salt was immediately deposited in characteristic rosettes, which after washing with hot absolute alcohol and recrystallization from hot water melted at 203°C.

Preparation of Guanosine from Guanine Mononucleotide.—Guanine mononucleotide was heated with five parts of 2.5 per cent ammonia in an autoclave for 2 hours at 140°C. On cooling in the ice chest guanosine was deposited as a gelatinous mass. This was filtered on a Buchner funnel and recrystallized from hot water with the use of animal charcoal. Pure guanosine was obtained in long transparent needles which on analysis gave the following results.

I. 0.2382 gm. air-dried substance required 14.11 cc. H_2SO_4 (1 cc. = 0.0037 gm. N).
II. 0.8086 gm. heated at 120°C. lost 0.0905 gm.

	H_2O	N
Calculated	11.29	21.94
Found.		
I		21.92
II	11.19	

Adenine-Uracil Dinucleotide.—Specimens of adenine-uracil dinucleotide prepared as described were submitted to hydrolysis with 5 per cent sulfuric acid. The product gave no precipitate of guanine on the addition of ammonia. The solution was treated directly with magnesia mixture and the free phosphoric acid determined as magnesium ammonium phosphate. The final filtrate from magnesium ammonium phosphate gave the characteristic gelatinous silver-adenine precipitate upon treatment with ammoniacal silver solution; so that the particular structure of the compound as a dinucleotide composed of one pyrimidine group containing firmly bound phosphoric acid and one purine group containing a phosphoric acid radicle easily set free by hydrolysis with dilute mineral acid is thus fully established.[7] Analyses for total and partial phosphorus gave results consistent with this structure.

I. 0.6936 gm. dried in a desiccator gave 0.2442 gm. $MgNH_4PO_4.6H_2O$.
II. 0.7803 " " " " " " 0.2751 " " "
III. 1.2024 " " " " " " after complete destruction of organic matter 0.7762 gm. $MgNH_4PO_4.6H_2O$.

	I.	II.	III.
Substance used	0.6936	0.7803	1.2024
Time of hydrolysis	3 hrs.	3 hrs.	Total.
$MgNH_4PO_4.6H_2O$ obtained	0.2442	0.2751	0.7762
Amount calculated per gm.	0.3521	0.3535	0.6450
Pyrimidine correction	0.0300	0.0300	
From purine nucleotide	0.3221	0.3235	
Half the total phosphorus			0.3225

[7] For full discussion of this subject see Germann, H. C., *J. Biol. Chem.*, 1916, xxv, 189.

The above results show that exactly half of the phosphoric acid of this dinucleotide is easily split and the other half is firmly bound.

Preparation of Uridine from the Dinucleotide.—25 gm. of dinucleotide were dissolved in 125 cc. of 2.5 per cent ammonia and heated in an autoclave at 133°C. for 2 hours. The product was allowed to stand in ice water for several hours but no trace of guanosine was deposited.

A portion of the autoclave product was warmed and treated with a hot aqueous solution of picric acid until cold picric acid failed to give a precipitate with a drop of the fluid. Upon recrystallization of the bulky gelatinous precipitate from hot water, adenosine picrate was obtained in characteristic transparent yellow plates.

The main portion of the autoclave product was treated for uridine by the method of Levene and La Forge.[4] Snow-white crystalline uridine was obtained, which melted at 158° (corrected), and gave the following analysis.

0.3746 gm. air-dried substance required 11.57 cc. H_2SO_4 (1 cc. = 0.0037 gm. N).

	N
Calculated	11.48
Found	11.43

Brucine Salt of the Dinucleotide.—3 gm. of substance dissolved in 9 cc. of hot water were treated with a solution of 7.2 gm. of brucine in 15 cc. of hot absolute alcohol. After washing the precipitated brucine salt thoroughly with hot alcohol, it was recrystallized from fifty parts of hot water. The characteristic crystalline substance melted at 175°, and gave the following analytical data.

I. 0.2222 gm. heated at 115°C. lost 0.0228 gm.
II. 0.4300 " gave 33.2 cc. N at 757 mm. and 28°C.
III. 0.4458 " " 34.6 " " " 754 " " 27°C.
IV. 1.2110 " " on complete oxidation of all organic matter 0.1098 gm. $Mg_2P_2O_7$.
V. 1.3013 gm. gave on complete oxidation of all organic matter 0.1189 gm. $Mg_2P_2O_7$.
VI. 0.3867 gm. gave 0.2750 gm. crystalline brucine, $C_{23}H_{26}N_2O_4.4H_2O$ (no chloroform extraction was made of the mother liquor).

	H_2O	N	P	Brucine.
Calculated	10.16	8.46	2.49	75.1
Found.				
I	10.26			
II		8.44		
III		8.47		
IV			2.49	
V			2.55	
VI				71.1

THE PROTEOCLASTIC TISSUE ENZYMES OF THE SPLEEN.

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(Received for publication, June 11, 1917.)

Problem.—Two autolytic enzymes have been described in the spleen. The one is described (1–4) as being active in an alkaline medium, adsorbable by kieselguhr and extractible with 5 per cent NaCl solution. This is the α-protease of Hedin and his coworkers. The other enzyme which is capable of hydrolyzing proteins is the β-protease of these investigators, having the characteristics of being salted out with ammonium sulfate and of being extractible with dilute acetic acid. It is not adsorbed by kieselguhr.

The writer has attempted to determine the range of activity of autolyzing enzymes, and inasmuch as it appears that the spleen varies from other tissues in regard to the relation between enzyme activity and hydrogen ion concentration (5), a study has been made concerning this relation in spleen enzymes.

Methods.

Spleens from the ox and the dog were used; the former, obtained from the hospital butcher, was about 48 hours old; the latter was prepared from a freshly killed dog. No characteristic differences in spleen enzymes were observed in the two cases.

The digests were prepared in the same manner in which they have been prepared in the former studies on tissue enzyme action (6). The tunica was removed before the spleen pulp was ground. Suspensions were made in the various phosphate mixtures given below so that 40 gm. net weight of spleen tissue were made up to 100 gm. by means of the phosphate mixtures. These mixtures were prepared from Sörensen's chart (7), the pH varying from 5.68 to 7.59 for beef spleen and to 7.98 for that of the dog. These mixtures were then read on a Leeds and Northrup Type K potentiometer. A 0.1 N calomel electrode, Leeds and Northrup standard cadmium element, and a Clark (8) oscillating hydrogen vessel with platinum electrode were

used. The hydrogen was obtained electrolytically and purified by means of a palladium sponge filter of de Khotinsky.[1]

α-Amino nitrogen determinations were made by means of the gasometric method of Van Slyke. Aliquots were used from the filtrates of 2.5 per cent trichloroacetic acid precipitates for these determinations.

Reaction.	Amino nitrogen per 40 gm. of fresh tissue.		
	Initial.	24 hrs.	6 days.
pH	*gm.*	*gm.*	*gm.*
5.68	0.221*		0.343
6.55	0.221		0.322
6.58	0.221		0.290
6.80	0.221		0.268
6.94	0.221		0.238
7.08	0.221		0.238
7.59	0.221		0.211
7.98	0.0053**	0.0053	0.0055

* Beef.
** Dog.

DISCUSSION.

It will be observed that digestion occurs where pH<7.08. pH 7.07 is approximate neutrality at the temperatures at which these readings were made. A departure from neutrality towards alkalinity results in inhibition of hydrolysis *as far as the proteins of the tissue are concerned.* Apparently the enzyme or enzymes are active at an alkalinity of pH 7.5–7.9, as will be seen from the following experiment where 5 gm. of Witte's peptone were introduced into a digest composed as the one given above for dog spleen in pH 7.98 phosphate mixture.

	Reaction.	Amino nitrogen per 40 gm. of tissue.	
		Initial.	24 hrs.
	pH	*gm.*	*gm.*
Control	7.98	0.054	0.054
" + 5 gm. of peptone	7.98	0.107	0.217

[1] Through the generosity of Mr. Albert Kuppenheimer of Chicago the Institute has been able to procure physicochemical apparatus of various sorts. The present work was done by means of the apparatus for electromotive force, purchased from this fund.

The peptone is digested to a marked extent, showing that a protein-hydrolyzing enzyme (or enzymes) is active. It is difficult to explain, however, why, if the enzymes are active at neutrality and in an alkaline reaction, no increase in α-amino nitrogen is obtained from the polypeptides which occur in partly autolyzed tissue, such as that obtained 48 hours after the animal's death; for the writer has determined that peptones and higher amino-acid linkages occur to a considerable extent in liver from a meat market. On adding large amounts of peptone and albumose, as in these experiments, a correspondingly large increase in amino nitrogen is obtained over that which is present initially. The conclusion is unescapable that native peptones and other polypeptides are not hydrolyzed by the tissue enzymes under the conditions of these experiments, although those introduced are digested.

Inasmuch as these experiments show that as far as the native proteins of the spleen are concerned, no alkaline-acting enzyme is recognizable capable of hydrolyzing these proteins, the experiments are divergent from those of Hedin and others who have described an enzyme of this nature, the so called α-protease. The question arises as to whether the α-protease is a true autolytic enzyme, belonging to the spleen tissue, or whether it is not of the nature of Martin Jacoby's *heterolytic* enzyme, and is derived from the leukocytes which abound in the spleen. For these bodies, alkaline-acting enzymes have been described by Opie (9) and later by Jobling and Strouse (10). Leukoproteases have been described by Leber (11), Jochmann and Ziegler (12), and others, following the initial work of Müller (13), but the range of their activity in regard to reaction of medium is not known.

CONCLUSIONS.

An enzyme or enzymes exist in the spleen, capable of hydrolyzing peptone and also (as shown by Hedin) fibrin. The proteins of the spleen itself, however, autolyze only in neutral or acid, not in alkaline solution. The most rapid autolysis was observed in the most acid solution tested, pH = 5.68.

From the standpoint of necrosis, the α-protease of Hedin can scarcely be operative since there is no evidence that it affects native proteins.

It is probable that the α-protease of Hedin is not an autolytic enzyme, but rather a heterolytic one, resident in the white blood cells, for such an enzyme has been described, definitely, for such cells.

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COMPARATIVE METABOLISM OF CERTAIN AROMATIC ACIDS.

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Fate of Phenylacetic Acid in the Organism of the Monkey.

Many experiments have been reported showing a marked difference between metabolism in the human body and in the lower animals. This alone has stimulated considerable interest on account of the apparent lack of reason for such difference. In the last few years experiments have been carried out with animals more closely related to the human in order to compare their metabolism with that of both human beings and lower animals.

Hunter and Givens (1) report the purine metabolism in the monkey as resembling that of the lower animals and not the human. In the urine of the monkey (*Cercopithecus*) allantoin accounted for 73 per cent of the nitrogen arising from the catabolism of the endogenous purines while the remainder appeared principally as purine bases and none as uric acid. Later work by the same authors (2) shows like results for another monkey (*Cercopithecus callitrichus*). Wiechowski (3) obtained results comparable to those of Hunter and Givens but was unable to find allantoin as the end-product of the purine metabolism of the chimpanzee. Baumann and Oviatt (4) found the urinary sulfur excretion in the monkey (*Macacus*) quite different from that of man. In the case of the monkey the ratio of inorganic to ethereal sulfates was approximately $3\frac{1}{2}$ to 1 while for man it bore the ratio 12 to 1.

An interesting phase of this same question is the manner in which the different animal organisms detoxicate certain protein putrefactive products. The *series of compounds* resulting from the action of putrefactive bacteria on the aromatic amino-acids must be detoxicated and eliminated from the animal body as

soon as possible. This detoxication process seems often to differ for different species or at least results in the excretion of an entirely different end-product for the acid thus resorbed.

Phenylacetic acid is one of those aromatic acids which yield different conjugation compounds in the urine after ingestion by men and animals respectively. Phenylacetic acid in combination with glycocoll is excreted normally by many of the herbivora as phenaceturic acid. Phenaceturic acid was discovered by Salkowski (5) in normal horse urine. Phenaceturic acid has been found in the urine of many different animals after feeding phenylacetic acid. Thus E. and H. Salkowski (6) isolated it from the urine of dogs and rabbits, and Vasiliu (7) from the urine of sheep after phenylacetic acid feeding. E. Salkowski (8) believed he had found traces of the phenaceturic acid in normal human urine. Totani (9) isolated from the excreta of chickens phenylacetylornithuric acid after phenylacetic acid feeding. Thierfelder and Sherwin (10) found in human urine two compounds, phenylacetyl glutamine and phenylacetyl glutamine urea after various amounts of phenylacetic acid had been ingested but were unable to find even a trace of either phenaceturic acid or uncombined phenylacetic acid.

Before beginning certain metabolism work on a monkey it was important to know whether the fate of the phenylacetic acid in the organism of the monkey would resemble that of the human organism or that of the lower animals. The combination with glycocoll resulted showing the metabolic process to resemble that of the lower animals, not only in the case of the phenylacetic but also for the *p*-hydroxyphenylacetic and *p*-hydroxy benzoic acid (11).

EXPERIMENTAL.

A 4.2 kg. female monkey (*Macacus rhesus*) was placed on a regular diet of milk, bread, bananas, and apples for several days in order to see if the urine contained traces of any phenylacetic acid compound. After no compound of this nature could be found in the urine, the monkey was fed 1 gm. of phenylacetic acid per day as the soluble sodium salt. The sodium salt dissolved in fresh milk at first seemed quite acceptable, but after the 3rd day it was refused. Any food or liquid possessing even the slightest odor of phenylacetic acid was entirely refused by the monkey. At this point forced feeding was resorted to and the water solution of the sodium salt introduced directly into the stomach by means of a stomach tube. The

amount thus given varied between 1 and 2 gm. The physiological effect seemed to be much the same as that produced on human beings. Each dose was followed by a period of dullness and inactivity, while the ingestion of 2 gm. (0.47 gm. per kg. body weight) resulted in an entire loss of appetite for 24 hours. When the phenylacetic acid is ingested by men in quantities varying from 0.05 to 0.26 gm. per kg. body weight intoxication results much the same as that produced by alcohol.

The monkey's urine was collected for 36 hours after each dose of the acid, and the different portions were united and acidified with phosphoric acid until a distinct acid test with Congo red resulted. The concentrated urine was then extracted repeatedly in a liquid extracting apparatus with ethylacetate until no phenylacetic acid compound formed by evaporation of the last extraction. The ethylacetate extract thus prepared was evaporated to one-half its original volume and placed on ice for 24 hours. As no phenylacetyl glutamine crystallized out at this concentration, the extract was again evaporated and the evaporation continued on subsequent days until the entire ethylacetate solution amounted to about 100 cc. only. At this concentration, there being no possibility of obtaining any phenylacetyl glutamine, the extract was evaporated carefully almost to dryness and then taken up with 50 cc. of water and boiled with charcoal to remove the pigment. After filtering this water solution from the charcoal, evaporating, and allowing the concentrated solution to stand on ice, there appeared small rhombic crystals of phenaceturic acid. The mother liquor from these crystals was optically inactive, showing again the absence of phenylacetyl glutamine. After the feeding of 1 gm. of phenylacetic acid the amount of phenaceturic acid isolated from the urine amounted to only 0.82 gm. or 51 per cent of the expected yield.

The phenaceturic acid was twice recrystallized from water, dried *in vacuo*, and then used for analyses.

Melting point 142–143°.

0.1232 gm. of the acid required 6.29 cc. of 0.1 N sodium hydroxide instead of 6.37 cc., the theoretical amount.

0.1331 gm. substance gave 8.50 cc. nitrogen at 21° and 748 mm. pressure.

	Calculated for $C_{10}H_{11}NO_3$:	Found:
N	7.25	7.15

CONCLUSION.

The metabolism of phenylacetic acid in the organism of the monkey is the same as that found in the lower animals and entirely different from the metabolism of the same substance in man.

Phenylacetic acid in the monkey is conjugated with glycocoll and excreted as phenaceturic acid, while in man it is conjugated with glutamine and excreted partly as phenylacetyl glutamine and partly as phenylacetyl glutamine urea.

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STUDIES ON BIOLUMINESCENCE.

VIII. THE MECHANISM OF THE PRODUCTION OF LIGHT DURING THE OXIDATION OF PYROGALLOL.

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INTRODUCTION.

It has been very definitely established from the observations of many investigators that the production of light by animals is a chemiluminescent phenomenon; *i.e.*, a luminescence accompanying a chemical reaction. As free oxygen is necessary for light production in all forms, the reaction may be classed among the oxidations. In several animals two substances (Harvey, 1) in addition to water and oxygen are necessary to produce light and at least one of these is oxidized. As a number of compounds of definite chemical composition are known which emit light during oxidation, it seemed desirable to study such reactions more in detail in order to determine under what conditions the light is emitted.

In a previous paper (Harvey, 1916) I showed that pyrogallol in weak solutions would produce light if oxidized by the plant peroxidases or by blood containing hemoglobin or by certain salts ($KMnO_4$, $K_4Fe(CN)_6$, and $FeCl_3$) if H_2O_2 were present. These substances may be collectively spoken of as oxidizers. No light appeared in the absence of H_2O_2 and no light appeared with pyrogallol and H_2O_2 alone. All means of oxidizing pyrogallol with light production were also efficacious in blueing gum guaiac, but guaiac can be oxidized by many methods which fail to produce light with pyrogallol. The oxidation of gum guaiac was never observed to produce light nor was light ever observed during the oxidation by peroxidases of many other easily oxidizable hydroxy-phenyl and amino-phenyl compounds. No other peroxide was found that could take the place of H_2O_2 in the luminous oxidation of pyrogallol by plant peroxidases. The peroxidase unquestionably acts by transferring oxygen from H_2O_2 to the pyrogallol, and both H_2O_2 and peroxidase are used up in the process (2). It is not necessary that H_2O_2

be decomposed with formation of bubbles of oxygen. KCN, acids, alkalies, and heat affect the light production in a characteristic way because they affect the peroxidase. It is consequently necessary, in order to gain a more accurate knowledge of the conditions under which the oxidation of pyrogallol leads to light production, to employ oxidizing substances of definite composition which are not affected by temperature, acid, etc. The present paper deals with the production of light by these inorganic substances.

The first observation that pyrogallol would luminesce was made in connection with photography. Lenard and Wolf (6) noticed that a photographic plate which was developed with a pyrogallol—Na_2SO_3—K_2CO_3 developer glowed, if dipped in a saturated alum solution. They found also that the developer itself would light on mixing with alum solution, and thought that light was due to pyrogallol, which collected about the $Al(OH)_3$ precipitated by K_2CO_3 and was there oxidized. $K_2S_2O_5$ might replace the Na_2SO_3 of the developer (21).

Trautz and Schorigin (8) also observed the luminescence of a photographic plate as described by Lenard and Wolf and noted in addition that 30 per cent formaldehyde might take the place of the saturated alum solution. In addition they noticed that a piece of raw leather, extracted with ligroin to remove fat, will luminesce if moistened with 30 per cent formaldehyde and exposed to the air. Further work showed that both aldehydes and phenol derivatives luminesce when oxidized in many ways, as follows.

Aldehydes.

1. With (*a*) neutral or (*b*) alkaline H_2O_2.

   (*a*) CH_2O (35 per cent) + H_2O_2 (3 to 30 per cent) = $CO_2 + H_2O + H_2$.

   (*b*) $2CH_2O + 2NaOH + H_2O_2 = 2\ HCOONa + 2\ H_2O + H_2$.

(Acetic, propionic, valeric, benzoic, and salicylic aldehydes and glucose give light under the same conditions.)

2. With Na_2O_2 in ice water (formaldehyde, acetaldehyde, glucose, and vanillin).

3. With a warm saturated solution of $NaClO_3$ (formaldehyde).

4. With alkalies (simple aldehydes, sugars, alcohols, and many other substances).

Phenols.

1. With warm neutral H_2O_2 or alkaline H_2O_2 at room temperatures (pyrogallol, tannic, and gallic acids, benzkatechin, metol, eikonogen, and naphthol).

2. With alkalies (pyrogallol, tannic, and gallic acids and many others).

According to Trautz and Schorigin, when 2 cc. of 10 per cent pyrogallol are mixed with 5 cc. of 10 per cent Na_2CO_3, the solution glows at the surface in contact with air; if some formaldehyde is added to this, the light lasts a long time, and if H_2O_2 is also added the light is much brighter. Trautz' well known luminescent reaction with pyrogallol results when 35 cc. of 50 per cent K_2CO_3, 35 cc. of 10 per cent pyrogallol, 35 cc. of 35 per cent

formaldehyde, and 50 cc. of 30 per cent H_2O_2 are mixed. A reddish glow appears, whose tint is no doubt due to the rapidly formed dark brown oxidation products of the pyrogallol. It is not necessary to use concentrated H_2O_2, as I have obtained a beautiful glow with 2 cc. of pyrogallol (12.5 per cent), 5 cc. of 10 per cent Na_2CO_3, 2 cc. of 40 per cent formaldehyde, and 1 cc. of 3 per cent H_2O_2. When the luminescence has faded, light again appears if more 3 per cent H_2O_2 is added. In my experience, Na_2CO_3 and pyrogallol alone, or pyrogallol + Na_2CO_3 + 3 per cent H_2O_2, or pyrogallol + formaldehyde, or pyrogallol + formaldehyde + 3 per cent H_2O_2, or pyrogallol + 3 per cent H_2O_2 gave no light. As we shall see, the proportions of substances and temperature greatly influence the light and my failure to observe light with pyrogallol and Na_2CO_3 alone may possibly be explained on this basis.

Trautz and Schorigin found also that hydroquinone and resorcinol, but not phenol would luminesce under the same conditions as pyrogallol. The light was stronger the greater the concentration of (1) the pyrogallol, (2) aldehyde, (3) alkali, or (4) H_2O_2, and (5) the higher the temperature. To give the brightest light the relation between aldehyde and pyrogallol should be: CH_2O : pyrogallol = (5–10) : 1. With the same substance, the time the light lasts is longer the weaker the light. These results suggest that reaction velocity is the factor determining the production of light, and Trautz (9) is fully convinced that such is the case. My own observations on temperature and concentrations of reacting substances to be described below do not wholly bear this out.

Many other types of substances besides aldehydes and derivatives of phenols can luminesce. Such cases have been collected and added to in the extensive paper by Trautz (9) and include among organic compounds acetylene derivatives, aldehydes, monobasic saturated primary alcohols, monobasic fatty acids, polyphenols, substances with ammonia residues, and substances with condensed benzene nucleus.

Among these are found many compounds of biological interest; *i.e.*, many products of plants or animals, as the following: terpene or fatty oils and waxes, glucose, lecithin, cholesterol, cholic, taurocholic, and glycocholic acids, cetyl and myricyl alcohol (Radziszewski, 10); benzaldehyde and vanillin, tannic and gallic acids, glycerol, mannite, papaverin (Trautz, 8, 9); uric acid and asparagine (Guinchant, 11); esculin (Dubois, 12); substances in humus (Weitlaner, 18); and substances in urine, the anaerobic alkaline hydrolysis products of glue, and Witte's peptone (McDermott, 13).

The various types of chemiluminescent reactions of general or biological interest may be grouped as below. The list also includes types described in the present paper.

1. Oxidation in air spontaneously.
   (*a*) At ordinary temperatures. Phosphorus. Fresh cut surfaces of Na or K.
   (*b*) At melting or vaporizing points. Fats, terpenes, sugars, resins, gums, ether, silk, and others.

2. Neutralization of strong bases and acids. CaO or BaO with concentrated H_2SO_4 or water. KOH with strong acids. $Ca(OH)_2$ and perchloric acid.
3. Decomposition of H_2O_2. If dropped on Ag_2O, Pb_3O_4, PbO_2, MnO_2, Ag, Pt, or Os.
4. Oxidation in aqueous or alcoholic alkalies. Many organic substances.
5. " " hypoiodites, hypobromites, or hypochlorites. Many organic substances.
6. Oxidation in peroxides (H_2O_2 or Na_2O_2). Many organic substances.
7. " " ozone.
8. " " acid permanganate. Pyrogallol (see p. 331).
9. " " persulfates and perborates. Formaldehyde, paraformaldehyde.
10. Oxidation in perchlorates, periodates, and perbromates. Palmitic acid.
11. Combination of 4 and 6. Many organic substances.
12. " " 5 " 6. " " "
13. " " 8 " 6. " " "
14. " " 5 " 9. " " "
15. Oxidation with H_2O_2 and hemoglobin or vegetable oxidases. Pyrogallol, lophin, esculin.
16. Oxidation with H_2O_2 and MnO_2, $Fe_2Fe(CN)_6$, $Mn(OH)_2 + Mn(OH)_3$, Ag_2O. Pyrogallol (Table I).
17. Oxidation with H_2O_2 and ferrocyanides, chromates, bichromates, permanganates, Fe salts, and Cr salts. Pyrogallol.
18. Oxidation with H_2O_2 and colloidal Ag and Pt. Pyrogallol (Table I).

In studying luminescence, Trautz and other observers have worked with concentrated solutions. It is not only unnecessary to use concentrated solutions to obtain light but concentrated solutions are to be avoided in many cases. For instance, M pyrogallol (12.6 per cent) will give no light if mixed with M/20 potassium ferrocyanide and some 3 per cent H_2O_2, but M/100 pyrogallol will give a good light, M/1,000 pyrogallol will give a brighter light, and M/100,000 pyrogallol will give a just visible light. We should expect to obtain a brighter light the greater the concentration of the pyrogallol, yet such is not the case. We should also expect to obtain a brighter light the greater the concentration of the $K_4Fe(CN)_6$ but neither is that the case. M/2 $K_4Fe(CN)_6$ will give no light with a mixture of M/100 pyrogallol and 3 per cent H_2O_2, whereas M/20 $K_4Fe(CN)_6$ does. The present paper deals with such anomalous phenomena as the above and with the mechanism of light production by pyrogallol in general. Additional substances have also been discovered which give light with

pyrogallol. The subjects treated can be conveniently considered under the following heads.

1. Oxidizers giving light with pyrogallol plus hydrogen peroxide.
2. Luminescence during electrolysis.
3. Peroxides giving light with pyrogallol and oxidizer.
4. Concentration of pyrogallol and oxidizer and light production.
5. Temperature and light production.
6. Light production in absence of peroxide.
7. Light production in non-aqueous solvents.
8. Effect of acid and alkali on light production.

EXPERIMENTAL.

1. Oxidizers Giving Light with Pyrogallol plus Hydrogen Peroxide.—Many substances in addition to those enumerated in my former paper have been found to oxidize pyrogallol with light production, but only if H_2O_2 is also present. As mentioned above, the concentration of pyrogallol has such a marked influence on light production that in the following experiments the tests were always made by mixing equal parts of M/100 pyrogallol and neutral 3 per cent H_2O_2, and adding to this an equal amount of the solution to be tested. The results are collected in Table I.

The substances tested can be divided into three groups according as they are present in true solution, in colloidal solution, or as precipitates. The concentrations given indicate those tried, in the case of substances giving no light, or the concentration giving the best light, in the case of substances which do give light. The power to blue guaiac alone or with H_2O_2 and the decomposition of H_2O_2 are also recorded.

Among the salts in solution, the ferrocyanides, chromates, bichromates, permanganates, iron and chromium compounds, and hypochlorite, hypoiodites, and hypobromites give light. Among the precipitates, $Fe_2Fe(CN)_6$, Ag_2O, and manganese dioxides, oxides, and hydroxides give light. Among the colloidal substances, in addition to the natural oxidizers in the blood of vertebrates and certain worms (hemoglobin), certain crustacea and mollusks (hemocyanin?), and plant juices (peroxidases), colloidal silver and platinum, and colloidal manganese compounds (possibly $Mn(OH)_3$) formed on the addition of $KMnO_4$ to proteins and organic substances gave light. In general, these compounds have a marked power of blueing guaiac, alone or with H_2O_2, but many

TABLE I.

Substances Giving Light with Pyrogallol and Hydrogen Peroxide.
True Solutions.

Equal volume added to mixture of 1 part M/100 pyrogallol or 1 part 3 per cent H_2O_2 + 1 part M/100 pyrogallol; hence, concentrations in final mixture are one-half that given.		Light with pyrogallol.	Light with pyrogallol + H_2O_2.	Blueing of gum guaiac.	Blueing of gum guaiac + H_2O_2.	Liberation of oxygen from H_2O_2.
1	Potassium ferrocyanide ($K_4Fe(CN)_6$ M/10–M/20)	–	Bright.	+		+
2	" ferricyanide ($K_3Fe(CN)_6$ M/10–M/1,250)	–	Very faint to –.	–	–	Very slow.
3	" chromate (K_2CrO_4 M/20–M/100)	–	Good.	+		+
4	" bichromate ($K_2Cr_2O_7$ M/50–M/100)	–	"	+		+
5	" permanganate ($KMnO_4$ M/50–M/200)	–*	Bright.	+	–**	+
6	" hydroxide (KOH M–M/6,250)	–	–	–	–	Very slow.
7	" chlorate ($KClO_3$ M/10)	–	–	–	–	–
8	" persulfate ($K_2S_2O_8$ M/10–M/128)	–	–	–	–	–
9	" chromium alum ($Cr_2(SO_4)_3 \cdot K_2SO_4$ M/10)	–	Faint.	Very slow.	Very slow.	–
10	Ferric ammonium alum ($Fe_2(SO_4)_3 \cdot (NH_4)_2SO_4$ M/10)	–	"	+		Very slow.
11	" chloride ($FeCl_3$ M/10–M/250)	–	Fair.	+		Slow.
12	Ferrous sulfate ($FeSO_4$ M/10–M/6,250)	–	"	–	+	"
13	Copper sulfate ($CuSO_4$ M/5–M/125)	–	–	–	+	Very slow.
14	Chromic acid (CrO_3 M/100)	–	Bright.	+		+
15	" sulfate ($Cr_2(SO_4)_3$ 2 per cent)	–	Faint.	–	+	Slow.
16	Manganous chloride ($MnCl_2$ M/2–M/125)	–	–	–	Slow.	"
17	" acetate ($Mn(CH_3COO)_2$)	–	–	–	–	Very slow.

18	Chlorine water		−	−	+		+
19	Bromine "		−	−	+		+
20	Iodine in KI		−	−	+		+
21	Sodium hypochlorite	(Cl water + NaOH)	Faint flash.	Bright.	+		++
22	" hypobromite	(NaOBr, bromine water + NaOH)	"	"	+		++
23	" hypoiodite	(I in KI + NaOH)	−	Faint.	+		+
24	Calcium hypochlorite	($Ca(OCl)_2$ saturated solution)	−	Good.	+		++
25	Silver nitrate	($AgNO_3$ 2 per cent)	−	−	−	−	−
26	Molybdic acid	(MoO_3 saturated solution)	−	−	−	+	Very slow.
27	Sodium molybdate	(Saturated MoO_3 neutralized with NaOH)	−	−	−	+	+
28	Uranium acetate	($UO_2(C_2H_3O_2)_2$ M/20–M/2,500)	−	−	−	−	−

Colloidal solutions.

1	Turnip juice		−	Bright.	−	+	++
2	" " heated to 70°		−	Faint.	−	+	Very slow.
3	" " boiled		−	−	−	−	−
4	" " + $KMnO_4$		−	Bright.	+		++
5	" " + $KMnO_4$ to 70° and filtered, filtrate		−	Faint.	−	+	−
6	" " " " " " " precipitate		−	"	Slow.	+	+
7	" " " " boiled 1 min. and filtered, filtrate		−	−	−	−	−
8	" " " " " 1 " " " precipitate		−	Faint.	Slow.	+	+
9	" " boiled 1 min. + $KMnO_4$		−	Good.	+	−**	−
10	" " " 1 " " " to 65°C. and filtered		−	−	Slow.	−	−
11	" " " 1 " " " boiled again and filtered		−	−	−	−	−

* A momentary flash of light if permanganate is acid but no light in neutral or alkaline solution.

** If H_2O_2 is added to $KMnO_4$ the color disappears or if H_2O_2 is added to albumin, peptone, or gelatin + $KMnO_4$ the brown color disappears and then the blueing of guaiac will no longer take place.

TABLE I—*Continued.*

	Equal volume added to mixture of 1 part M/100 pyrogallol or 1 part 3 per cent H_2O_2 + 1 part M/100 pyrogallol; hence, concentrations in final mixture are one-half that given.	Light with pyrogallol.	Light with pyrogallol + H_2O_2.	Blueing of gum guaiac.	Blueing of gum guaiac + H_2O_2.	Liberation of oxygen from H_2O_2.
12	Albumin solution	−	−	−	−	−
13	" " + $KMnO_4$	−	Good.	+	−**	++
14	" " " " boiled 1 min. and filtered (no precipitate forms)	−	"	+	−**	++
15	Gelatin solution	−	−	−	−	−
16	" " + $KMnO_4$	−	Good.	−	−**	++
17	" " " " boiled 1 min. and filtered (no precipitate forms)	−	"	+	−**	++
18	Witte peptone solution	−	−	−	−	−
19	" " " + $KMnO_4$, filtered	−	Faint.	+	−**	++
20	" " " " " boiled 1 min., filtrate	−	−	−	−	−
21	" " " " " " 1 " precipitate	−	Faint.	Slow.	+	++
22	Colloidal Ag	−	Bright.	+		+
23	" Pt	−	"	+		+
24	" $Fe(OH)_3$ (dilute)	−	−	−	+	−
25	Sodium nucleoproteinate (liver)	−	−	−	+	−
26	" " (mammary gland)	−	−	−	−	−
27	" nucleate (yeast)	−	−	−	−	−
28	Squid blood (*Sepia esculenta*). Contains hemocyanin	−	Fair.			++
29	" " " " boiled	−	Good.			−
30	Lobster blood (*Palinurus japonicus*). Contains hemocyanin and tetronerythrin, a lipochrome	−	Faint.			++
31	" blood (*Palinurus japonicus*) boiled	−	Fair.			−
32	Annelid blood (*Laonome japonica*). Contains chlorocruorin	−	Good.			
33	" " " " boiled	−	−			

34	Annelid extract (*Nereis* sp.)	−	Fair.			++
35	" " (*Thelepus japonicus*)	−	Good.			++
36	" " (*Hesione reticulata*)	−	Fair.			++
37	*Chiton* sp. extract	−	"			++
38	" " " boiled	−	"			−
39	Luminous pennatulid extract (*Cavernularia haberi*)	−	−	−	+	++
40	" ostracod " (*Cypridina hilgendorfii*)	−	−			+
41	" protozoan " (*Noctiluca miliaris*)	−	−	−	−	−
42	Firefly (*Luciola viticollis*) extract, luminous organs	−	−			++
	Precipitates.					
1	Silver ferrocyanide ($Ag_4Fe(CN)_6$)	−	−	Slow.	+	Very slow.
2	Lead " ($Pb_2Fe(CN)_6$)	−	−	−	−	−
3	Ferrous " ($Fe_2Fe(CN)_6$)	−	Faint.	+		+
4	Zinc " ($Zn_2Fe(CN)_6$)	−	−	+		Very slow.
5	Copper " ($Cu_2Fe(CN)_6$)	−	−	+		+
6	Silver chromate (Ag_2CrO_4)	−	−	−	Very slow.	−
7	Lead " ($PbCrO_4$)	−	−	−	"	−
8	Barium " ($BaCrO_4$)	−	−	−	"	−
9	Chromic oxide (Cr_2O_3)	−	−	−	−	Slow.
10	" hydroxide ($Cr(OH)_3$)	−	−	−	Slow.	+
11	Manganese dioxide (MnO_2)	−	Good.	Slow.	"	++
12	Manganic and manganous hydroxide ($Mn(OH)_2 + Mn(OH)_3$)	−	"	"	+	+
13	Hydrated manganese hydroxide H_2MnO_3 (precipitated from $MnCl_2$ by Br + NaOH)	−	"	"	+	++

TABLE I—*Concluded.*

	Equal volume added to mixture of 1 part M/100 pyrogallol or 1 part 3 per cent H_2O_2 + 1 part M/100 pyrogallol; hence, concentrations in final mixture are one-half that given.	Light with pyrogallol.	Light with pyrogallol + H_2O_2.	Blueing of gum guaiac.	Blueing of gum guaiac + H_2O_2.	Liberation of oxygen from H_2O_2.
14	Manganic oxide (Mn_2O_3)	–	Faint.	"	+	++
15	Manganese metal (powder) (Mn)	–	–	"	Slow.	+
16	Metallic Ag	–	Faint.	+		++
17	Iron metal (Merck's reagent by hydrogen) (Fe)	–	–	–	–	Very slow.
18	Black ferrous oxide (FeO)	–	–	–	Slow.	+
19	Ferric hydroxide ($Fe(OH)_3$)	–	–	–	–	+
20	Ferrous-ferric oxide (Fe_3O_4)	–	–	–	–	+
21	Red ferric oxide (Fe_2O_3)	–	–	–	–	–
22	Copper hydroxide ($Cu(OH)_2$)	–	–	–	Slow.	+
23	Red copper oxide (Cu_2O)	–	–	Very slow.	+	+
24	Black " " (CuO)	–	–	–	+	+
25	Silver oxide (Ag_2O)	–	Faint.	+		+
26	Red mercuric oxide (HgO)	–	–	–	–	–
27	Black mercurous oxide (Hg_2O)	–	–	–	Slow.	+
28	Lead peroxide (PbO_2)	–	–	+		++
29	" oxide (PbO)	–	–	–	–	+
30	Red lead oxide (Pb_3O_4)	–	–	–	–	Very slow.
31	Bone black (C)	–	–	–	Very slow.	+
32	Infusorial earth (SiO_2)	–	–	–	–	–
33	Platinum black	–	Faint.	+		+

solutions which blue guaiac readily (bromine water) give no light with pyrogallol + H_2O_2, and some substances which blue guaiac + H_2O_2 only slowly (potassium chrom alum and MnO_2) give light with pyrogallol + H_2O_2. Generally the substances giving light with pyrogallol + H_2O_2 liberate visible bubbles of oxygen from H_2O_2 but this is not necessary for light production, as indicated by the plant extracts or blood which give a good light after the catalase has been destroyed. In my first paper I reported that blood containing hemocyanin gave no light but this is an error due to the use of solutions too much diluted. Note also from Table I that extracts of luminous animals give no light with pyrogallol.

Of special interest is the light produced by insoluble precipitates like MnO_2 and the colloidal metals Ag and Pt. Platinum black, with less surface, gives only a faint light. These exert a marked catalytic decomposition of H_2O_2 and they are said to be wholly unchanged at the end of the reaction. Indeed light has been observed when H_2O_2 is dropped upon finely divided MnO_2, Ag, or Pt (Dammer, 14) but with the concentrations of H_2O_2 used in my experiments light only appears if pyrogallol is also present. The Pt and Ag solutions were supplied by Dr. Goss of the Chemistry Department, whose paper on light production by colloidal solutions appears on page 271.

Potassium permanganate added to many organic substances is reduced to hydroxides, probably $Mn(OH)_3$, and with certain organic substances, especially proteins, the $Mn(OH)_3$ forms a colloidal solution. The colloidal $Mn(OH)_3$ has a marked oxidizing action. This is unquestionably the explanation of Reed's (16) supposed action of permanganate on peroxidases as indicated by the work of Bunzell and Hasselbring (15), a conclusion which I had already reached when their paper appeared. Reed supposed that the permanganate gave oxygen to the peroxidase converting it into a substance with greater oxidizing power, but the formation of colloidal $Mn(OH)_3$ explains his results satisfactorily. These colloidal Mn compounds will oxidize guaiac directly and give light with pyrogallol + H_2O_2 as will also a precipitate of $Mn(OH)_3$. My results are recorded in Table I. Although gum guaiac is oxidized without H_2O_2, no light appears with pyrogallol unless H_2O_2 is present. An excess of H_2O_2 converts the brown colloidal $Mn(OH)_3$ into colorless compounds which will then not blue guaiac.

It is a curious fact that although the ferrocyanides give a good light, the ferricyanides give none or at most a very faint light. Different samples of both ferrocyanide and ferricyanide solutions were found to vary in this regard and the variation proved to be the result of exposure of the solutions to light and air. Ferrocyanide particularly acquires the power to oxidize pyrogallol with bright light production after exposure to light and air. The phenomenon has nothing to do with phosphorescence (*i.e.*, the giving out of light rays after exposure to light) but is no doubt the result of the formation of some substance in the ferrocyanide by photochemical action. It is presumed that this substance, whatever it is, has a marked power of transferring oxygen from H_2O_2 to the pyrogallol. Photochemical changes in ferrocyanides and ferricyanides are well known and very marked and, according to Haber (22), consist in the splitting of $Fe(CN)_6''''$ to $Fe^{\cdot\cdot} + 6\ CN'$. Under the influence of light the iron separates and precipitates as $Fe(OH)_3$ or, if $(NH_4)_2S$ be added, as black FeS. However, other changes must also occur as neither iron salts nor $Fe(OH)_3$ in colloidal or precipitate form can oxidize pyrogallol with anything like the light production obtained with $K_4Fe(CN)_6$ after exposure to sunlight and air. The following tabulation gives the results of my light experiments with $K_4Fe(CN)_6$ and $K_3Fe(CN)_6$.

Solution.	Color before exposure to sunlight.	Color after exposure to sunlight 1 week.	Light production with M/100 pyrogallol + 3 per cent H_2O_2.	
			Solution exposed to sunlight.	Solution kept in the dark.
M/20 $K_4Fe(CN)_6$ in air.	Light yellow.	Deep yellow with brownish precipitate.	Bright.	Negative to very faint.
M/20 $K_4Fe(CN)_6$ in hydrogen.	"	Light yellow.	Negative.	"
M/20 $K_3Fe(CN)_6$ in air.	Yellow.	Dirty green with brown precipitate.	Fair.	"
M/20 $K_3Fe(CN)_6$ in hydrogen.	"	Dirty green with blue precipitate.	"	"

2. Luminescence During Electrolysis.—All attempts to obtain light from the oxidation of pyrogallol by the nascent oxygen set free at the anode during electrolysis have failed despite the fact that various oxidizers were present. Bancroft and Weiser (17) have described light at the anode during electrolysis of certain salts. An especially bright orange light was observed during the electrolysis of NaBr with the mercury anode. The color is the same as that obtained when mercury vapor burns in an atmosphere of bromine to form HgBr and the light during electrolysis is no doubt due to combination of the bromine set free by electrolysis with the mercury of the anode. A film of HgBr can be observed to form and under this film the mercury is supposed to be volatilized by the heating effect of the current.

Anode.	Electrolyte.	Light production at anode or cathode.
Platinized platinum.	Half saturated K_2SO_4 containing M/20 pyrogallol.	Negative.
" "	Half saturated K_2SO_4 containing M/200 pyrogallol.	"
" "	Turnip juice + K_2SO_4 containing M/200 pyrogallol.	"
Pt with PbO_2 surface.	Half saturated K_2SO_4 containing M/200 pyrogallol.	"
" " MnO_2 "	Half saturated K_2SO_4 containing M/200 pyrogallol.	"
Lead.	Half saturated K_2SO_4 containing M/200 pyrogallol.	"
Platinum.	$MnSO_4$ solution containing M/200 pyrogallol.	"

Reed (3) found that platinum black only blues guaiac if first exposed to nascent oxygen by making it the anode in some electrolyte. He used dilute HCl as the electrolyte, a fluid open to the objection that chlorine, whose powerful oxidizing action is so well known, is also liberated at the anode. This objection can be eliminated by using Na_2SO_4 solution as electrolyte and I can confirm Reed's result. A platinized surface made anode in a solution of Na_2SO_4 and then washed in water will blue gum guaiac directly but loses this power if made cathode in the same solution and then washed in water. Many other metallic sur-

faces will blue guaiac if made anodes but none of these give light with pyrogallol if made anodes in electrolytes containing pyrogallol. The combinations in the table on page 323 were tried. The Pt cathode which becomes markedly alkaline was enclosed in a porous cup. The current varied up to a maximum of 2 amperes.

In some experiments the Pt cathode instead of being enclosed in a porous cup was rotated rapidly but in no case did light appear.

3. Peroxides Giving Light with Pyrogallol and Oxidizers.—The peroxidase of turnip juice or $K_4Fe(CN)_6$ or any substance which gives light with pyrogallol and H_2O_2 may be spoken of collectively as oxidizers. No substance of peroxide nature other than H_2O_2 will give light of any brilliancy or permanency with these oxidizers. Certain oxidizers will give a momentary light with such peroxides as Na_2O_2, BaO_2, or perborates and persulfates but it is very faint and does not last as does the light with H_2O_2. The substances tried are listed in Table II.

TABLE II.

Peroxides Giving Light with Pyrogallol and Oxidizers.

Oxidizer. (Equal parts added to a mixture of M/100 pyrogallol and the peroxide.)	H_2O_2 3 per cent.	Benzoyl hydrogen peroxide (insoluble powder).	Ozonized turpentine (one drop).	Na_2O_2 (powder).	BaO_2 (powder).	MnO_2 (insoluble powder).	PbO_2 (insoluble powder).	K persulfate M/10.	Na perborate M/20.	K perchlorate M/10.	Quinone (insoluble crystals).
Turnip juice	+	–	–	–	–	–	–	–			–
1 per cent blood extract.	+	–	–	Faint flash.	–	–	–	–	–	–	–
M/20 $K_4Fe(CN)_6$	+	–	–	–	–	–	–	–	–	–	–
M/100 $KMnO_4$	+	–	–	–	–	–	–	Faint flash.	Fair flash.	–	–
M/10 $FeCl_3$	+								–	–	
M/100 CrO_3	+								–	–	
Na hypobromite	+	–	–	Faint flash.	Faint flash.	–	–	Fair flash.	Fair flash.	–	–
Ca hypochlorite	+	–	–	–	–	–	–	Faint.	"	–	–
MnO_2	+										
$Mn(OH)_3$ sol in peptone	+								–	–	
Colloidal Ag	+										

4. Concentration of Pyrogallol and Oxidizer and Light Production.—Pyrogallol and H_2O_2 and the oxidizer must naturally be present in a definite low concentration before light is visible. This concentration is surprisingly low for pyrogallol—M/32,000 when potato juice is used as oxidizer (Harvey, 1) and M/512,000 with $K_4Fe(CN)_6$. The latter dilution corresponds to 1 part of pyrogallol in 5,000,000 parts of water. With increasing pyrogallol concentrations the intensity of the light increases up to a limit and then decreases. In the case of vegetable peroxidases this is no doubt due in part to precipitation of the peroxidase, as a visible coagulum is formed by strong pyrogallol in potato juice. However, this explanation does not hold for $K_4Fe(CN)_6$. In the case of this oxidizer, we also find no light produced if too strong pyrogallol is used. It is also necessary to employ an optimum concentration of $K_4Fe(CN)_6$ in order to produce light. Too *great* as well as too small a concentration of $K_4Fe(CN)_6$ fails to excite luminescence. H_2O_2 must be present in a certain low concentration but there is apparently no upper limit in this case, at least to a concentration (after mixing) of 7.5 per cent. The actual concentrations necessary are given in Tables III and IV. An

TABLE III.

Concentration of Pyrogallol and Light Production with (after Mixing) 0.75 per Cent H_2O_2 and M/40 $K_4Fe(CN)_6$.

Concentration of pyrogallol (after mixing).	Character of light from mixture.
M/4	Negative.
M/40	Faint.
M/400	Good.
M/4,000	Bright.
M/8,000	"
M/16,000	"
M/32,000	Fair.
M/64,000	Faint.
M/128,000	"
M/256,000	Very faint.
M/512,000	Extremely faint.
M/1,024,000	Negative.

TABLE IV.

Concentration of $K_4Fe(CN)_6$ and Light Production with (after Mixing) 0.75 per Cent H_2O_2 and M/400 Pyrogallol.

Concentration of $K_4Fe(CN)_6$ (after mixing).	Character of light from mixture.
M/4*	Negative to very faint.
M/8	Faint.
M/16	Good.
M/32	"
M/64	Fair.
M/128	"
M/256	Faint.
M/512	Negative.

* If this solution is first exposed to sunlight a good light results.

excess (at least ten times the amount necessary) of H_2O_2 was present.

What is the explanation of this absence of light with concentrated pyrogallol or concentrated $K_4Fe(CN)_6$ solutions? We know that oxidation of the pyrogallol goes on because the mixture turns dark. One immediately thinks of absorption of light by the concentrated solutions. It is obvious that no light will be visible unless the intensity of emission is greater than the absorption. Both M pyrogallol and M/2 $K_4Fe(CN)_6$ are colored, the former a dirty light yellow, due to slight spontaneous oxidation, and the latter a clear light yellow. Neither of these solutions absorb light sufficiently to obscure the luminescence of mixtures of M/200 pyrogallol and M/20 $K_4Fe(CN)_6$ with H_2O_2, as we can very easily determine by viewing the luminescent mixture in a test-tube of 12 mm. diameter through at least a 40 mm. layer of M pyrogallol or M/2 $K_4Fe(CN)_6$. Only when the light of the luminescent mixture has markedly faded is the absorption noticeable. The oxidation products of pyrogallol, brown in color, do absorb light to a marked extent and the rapid fading of the luminescence of pyrogallol is no doubt largely due to the continued formation of these products. Absorption by brown oxidation products, which are formed only slowly, could not explain the *absence of immediate light production* on mixing M pyrogallol with H_2O_2 and M/20 $K_4Fe(CN)_6$ or M/2 $K_4Fe(CN)_6$ with M/100 pyrogallol and H_2O_2.

The explanation must lie in some other direction. Perhaps the greatest amount of light is emitted with a definite optimum velocity of oxidation of pyrogallol. The velocity of oxidation of pyrogallol should increase with increasing concentration of pyrogallol or of $K_4Fe(CN)_6$. We may suppose that with concentrated pyrogallol or concentrated $K_4Fe(CN)_6$ the velocity is above the optimum and although much heat is produced, none of the energy goes to light. If this supposition is correct we might expect to obtain light with concentrated pyrogallol or ferrocyanide by lowering the temperature, since we might in this way lower the reaction velocity to the desired optimum. Experiment has shown, however, that not lowering but on the contrary *raising* the temperature results in the greater production of light when either pyrogallol or ferrocyanide is present in *high concentration*. This

can be seen by a glance at Tables VI and VII. The phenomenon is akin to that observed during the oxidation and luminescence of phosphorus (23). White phosphorus will only luminesce in presence of oxygen but if the oxygen pressure is too great, the luminescence ceases. At 0°C. with water vapor present this "maximum luminescence pressure" of oxygen is 320 mm. Hg. and it increases 13.19 mm. Hg. for each degree rise in temperature. Thus there is a maximum and a minimum concentration of oxygen for the luminescence of phosphorus. In the case of pyrogallol we have a maximum and a minimum concentration of pyrogallol and also of the oxidizer for the luminescence of pyrogallol. There appears to be no maximum for H_2O_2. The effect of temperature is to raise the maximum and is the same both as regards phosphorus and pyrogallol.

The oxidation of phosphorus probably proceeds in several steps with the formation of intermediate oxides. If we assume that only one of these intermediate oxidations is connected with the production of light it is probable that a certain oxygen pressure and temperature will favor that reaction step at the expense of the others. This oxygen concentration and temperature will then correspond to the optimum for luminescence. A similar explanation may be applied to the oxidation of pyrogallol but this case is further complicated by the presence of an oxidizer.

Potassium ferrocyanide is not the only oxidizer which fails to give light with concentrated pyrogallol. Many others are known. These include M/100 $KMnO_4$, MnO_2 powder, M/100 CrO_3, and M/10 $FeCl_3$. On the contrary, NaClO and NaBrO give a bright flash no matter what the concentration of pyrogallol. If ozone gas is bubbled through M/100 pyrogallol (without H_2O_2) each bubble is brightly luminescent; with M/10 pyrogallol the bubbles are faintly luminescent, while no light appears if the pyrogallol is of M concentration. Pyrogallol behaves toward most of these oxidizers as toward $K_4Fe(CN)_6$ but the hypochlorites and hypobromites offer a marked exception for which no explanation is at present attempted.

5. Temperature and Light Production.—The effects of temperature have already been briefly mentioned in the preceding section. Since the velocity of most chemical reactions is doubled or tripled with each 10° rise in temperature, it is to be expected that the

TABLE V.

Temperature and Light Production. The Oxidizer Is Mixed with an Equal Amount of M/100 *Pyrogallol + 3 per Cent* H_2O_2.

Oxidizer.	Temperatures.				
	0–2°	20°	50°	75°	98–100°
Turnip juice	Faint.	Good.	Good.		Negative.
1 per cent blood extract	"	Fair.	"		Fair.
M/20 $K_4Fe(CN)_6$	Negative.	Good.	Bright.	Bright.	Good.
M/100 $KMnO_4$	Fair.	"	"	"	Faint flash.
M/50 $K_2Cr_2O_7$	Negative.	Fair.	Faint.	Fair.	Negative.
M/100 CrO_3	"	Good.	Bright.	Bright.	Faint.
M/10 KCr alum	"	Faint.	Faint.	Faint.	Negative.
M/10 NH_4Fe ' ' ' alum	"	"	"	"	Very faint.
MnO_2	"	Fair.	Fair.	Fair.	Negative.
NaClO	Bright flash.	Bright flash.	Bright flash.		Fair flash.

TABLE VI.

Temperature, Concentration of Pyrogallol, and Light Production. An Equal Amount of M/20 $K_4Fe(CN)_6$ *Is Mixed with Pyrogallol + 3 per Cent* H_2O_2.

Concentration of pyrogallol (after mixing).	Temperatures.						
	0–2°	10°	20°	30°	50°	75°	98–100°
M/4.....	Negative.	Negative.	Negative.	Very faint.	Faint.	Fair.	Faint.
M/40.....	"	Faint.	Faint.	Faint.	Good.	Bright.	Good.
M/400....	Faint.	Fair.	Good.	Good.	"	"	Bright flash.
M/4,000..	Bright.	Bright.	Bright.	Bright.	Bright flash.	Fair flash.	Negative.

TABLE VII.

Temperature, Concentration of Ferrocyanide, and Light Production. An Equal Amount of $K_4Fe(CN)_6$ *Is Mixed with* M/100 *Pyrogallol + 3 per Cent* H_2O_2.

Concentration of $K_4Fe(CN)_6$ exposed to light (after mixing).	Temperatures.						
	0–2°	10°	20°	30°	50°	75°	98–100°
Half saturated at 20°C.....	Negative.	Faint.	Fair.	Fair.	Good.	Good.	Faint flash.
One-sixth saturated at 20°C...	Very faint.	Fair.	Good.	Good.	Bright.	Very bright.	Good "

intensity of the light will be increased and the duration of the light decreased with rise in temperature. Although no numerical data can be given, my experiments do indicate that with rise of temperature, for each oxidizer (Table V), and each concentration of oxidizer (Table VII), or each concentration of pyrogallol (Table VI) the duration of the light is shorter and its intensity is greater up to a certain definite temperature. Above this temperature the intensity as well as the duration of the light decreases.

It is perfectly plain why potato juice gives no light at high temperatures, because its oxidizer is destroyed between 80° and 85°; but why should the light be fainter or absent at 98° in the case of $KMnO_4$ or MnO_2 or any of the oxidizers of Table V which are not affected by a temperature of 98°C.? In harmony with the explanation already suggested we must suppose that it is a definite (optimum) temperature and not the highest temperature which favors that particular step in the oxidation of pyrogallol involving the production of light. At the temperature indicated in the lower right hand square of Table VI, particularly, the oxidation proceeds in such a way that no light is produced.

6. Light Production in Absence of Peroxide.—When pyrogallol is oxidized by peroxidase and hydrogen peroxide, purpurogallin, a substance of doubtful composition, is said to be formed. Its formula is probably $C_{12}H_8O_5$ (4) or $C_{11}H_8O_5$ (5). This substance can be recognized qualitatively by its dark color. As purpurogallin is fairly insoluble in cold water, Bach and Chodat (2) determined it by collection on a weighed filter, drying at 110°, and again weighing. Reed (3) made determinations by filtering, washing free of unoxidized pyrogallol, and titrating by 0.05 M $KMnO_4$.

Purpurogallin or a similar substance is also formed when pyrogallol is oxidized by many oxidizing agents; *viz.*, by $AgNO_3$, $KMnO_4$, or $NaNO_2$ in acid solution, by quinone or platinum black or $K_3Fe(CN)_6$, or by allowing a solution of pyrogallol and gum arabic to stand. Perkin (5) reports a very favorable yield by the electrolytic oxidation of pyrogallol in 15 per cent Na_2SO_4 with lead cathode and rotating platinum anode. I have observed no light during the action of these substances in moderate concentrations (*i.e.*, in concentrations comparable to those previously described as giving light in the presence of H_2O_2) although the

pyrogallol turned brown. The $AgNO_3$ was reduced to metallic Ag. The following combinations were tried.

1 cc. 2 per cent $AgNO_3$ + 1 cc. N/10 HNO_3 mixed with 2 cc. M/100 pyrogallol.
1 cc. M/10 $NaNO_2$ + 1 cc. N/10 HNO_3 mixed with 2 cc. M/100 pyrogallol.
2 " " " mixed with 2 cc. M/10 pyrogallol.
1 " " $K_3Fe(CN)_6$ + 1 cc. N/10 HNO_3 mixed with 2 cc. M/100 pyrogallol.
Gum arabic solution and M/2 pyrogallol.

The gum arabic and pyrogallol mixtures become brown slowly (during the course of 2 or 3 days) and needle crystals, sometimes curved and forming rosettes, of a golden brown substance, separate. No light was observed to be given off during this process.

The only substances investigated capable of giving light with weak pyrogallol *alone* are sodium hypochlorite and hypobromite (Table I), ozone, and acid permanganate, which gave light in the following combination.

1 cc. M/100 $KMnO_4$ + 1 cc. N to N/10 HCl or HNO_3 mixed with 2 cc. M/100 pyrogallol.
Ozonized oxygen (by silent electric discharge) bubbled through M/100 pyrogallol.

The light with acid permanganate is at most a momentary flash. Neutral or alkaline permanganate gave no light with pyrogallol alone. Fahrig (19) had observed that ozone shaken with certain samples of water would luminesce but was unable to determine the cause of it. Otto (20) found that water carefully purified of organic matter gave no light with ozone but that benzene (feeble light), alcohol, thiophene, milk, and urine did. I find that M/100 and M/10 pyrogallol, M/10 orcinol, M/10 resorcinol, M/100 esculin, fresh or boiled potato juice, and certain other fluids give light if ozone is bubbled through them. Each bubble, as it rises through the liquid is aglow. As already mentioned, if the pyrogallol is too concentrated (M solution) no light appears. Pure oxygen gives no light if bubbled through the above solutions.

Pyrogallol also turns brown if an alkaline solution is exposed to air. CO_2, acetic acid, and brown substances are said to be formed. Oxygen is absorbed, the amount depending on the amount of alkali present up to a certain concentration. Weyl

and Goth (7) found that most oxygen was absorbed when 0.25 gm. of pyrogallol was present in 10 cc. of NaOH of specific gravity 1.03. Less pyrogallol or a greater or less amount of NaOH resulted in the absorption of less oxygen. Trautz and Schorigin (8) found that a mixture of 2 cc. of 10 per cent pyrogallol + 5 cc. of 10 per cent Na_2CO_3 luminesced at the surface but I have been unable to confirm this experiment or to observe light at room temperature during the darkening of pyrogallol + alkali under any conditions. Alkali of all concentrations from solid NaOH or Na_2CO_3 to N/6,250 NaOH has been added to various concentrations of pyrogallol but light has never been observed. It is perhaps not surprising to observe no light in the more concentrated solutions, which turn black almost instantly, because of absorption, but even in weak solutions (N/10 to N/6,250 NaOH + M/100 pyrogallol) *no light has ever been observed* even though H_2O_2 also be added. Such solutions become only brown in color, the more deeply brown the greater the concentration of the alkali.

The absence of light during the absorption of oxygen in alkaline pyrogallol is possibly due to the rapidity of the oxidation. The oxidation of the pyrogallol by $K_4Fe(CN)_6$ can be made to proceed so rapidly or in such a way that no light appears (Table VI, the 98° column); on the other hand it may be that the oxidation product formed with alkali is different from that formed with H_2O_2 and an oxidizer or that one step in the oxidation series is omitted. So little is known of the chemistry of purpurogallin that we cannot decide the question at the present time. It is apparent, however, that the conditions for light production are quite definite. The oxidation must proceed in a particular way.

7. *Light Production in Non-Aqueous Solvents. Alcohol.*—Light production occurs in fairly strong alcohol as indicated by the mixtures in Table VIII.

The turnip juice plus alcohol will give light with pyrogallol so long as the peroxidase is not precipitated but if precipitation occurs, then only a very faint light is produced. Strong alcohol (95 per cent), however, does not prevent light production so long as an oxidizer not affected by the alcohol, such as MnO_2, is employed. Even hemoglobin powder (Eimer and Amend) gives a faint light with pyrogallol plus H_2O_2 if suspended in absolute alcohol (No. 6 of Table VIII).

TABLE VIII.

Light Production in Alcohol.
Equal Parts of Solution A and Solution B Are Mixed.

No.	Solution A. 1 cc. of 3 per cent H_2O_2 in 90 per cent alcohol, plus:		Solution B.		Light production.
	1 cc. pyrogallol.	In alcohol.	Substance.	In alcohol.	
		per cent		*per cent*	
1	M/100	Absolute.	Precipitate formed by adding 90 per cent alcohol to turnip juice.	90	Very faint.*
2	M/200	50	Same with 50 per cent alcohol.	50	Faint.*
3	M/200	50	Filtrate from above precipitation.	50	Negative.
4	M/200	40	Precipitate formed by adding 40 per cent alcohol to turnip juice.	40	Bright.
5	M/200	40	Filtrate from above precipitation.	40	"
6	M/100	Absolute.	Hemoglobin powder.	Absolute.	Faint.
7	M/200	50	" "	50	Bright.
8	M/100	Absolute.	Powdered MnO_2.	Absolute.	Fair.
9	M/100	"	" H_2MnO_3.	"	Good.
10	M/100	"	M/10 $FeCl_3$.	"	Negative.
11	M/200	50	M/20 "	50	"

* Light appears only some time after mixing.

Acetone.—Hemoglobin or MnO_2 powder both give light with 50 per cent acetone containing M/200 pyrogallol and 1.5 per cent H_2O_2. The light from the hemoglobin is very bright. If benzoyl peroxide replaces the H_2O_2 no light appears.

Ether, Chloroform, and Benzene.—Pyrogallol is slightly soluble in benzene, fairly soluble in chloroform, and easily soluble in ether. If pyrogallol (M/100 or less) in ether, chloroform, or benzene be shaken with an equal volume of 3 per cent H_2O_2 in water, the ether, chloroform, and benzene layer then removed, and added to hemoglobin or MnO_2 powder, light only appears with ether and hemoglobin, and it is very faint. Benzoyl peroxide in place of H_2O_2, although soluble in all three solvents, will give no light in any case.

8. Effect of Acid and Alkali on Light Production.—The influence of acid and alkali on light production can best be studied by using as an oxidizer some inert substance such as MnO_2 not changed by acid or alkali in dilute concentration. Table IX gives the results. The MnO_2 powder was added to the HCl and NaOH in the concentrations indicated in the table and mixed with an equal volume of 1 cc. of 3 per cent H_2O_2 + 1 cc. M/100 pyrogallol. The concentration of acid and alkali in the final mixture is therefore one-half of that designated in the table. It will be observed that a small concentration of acid (N/160) inhibits the light, whereas a definite concentration of alkali (N/40) increases the light to an optimum.

TABLE IX.

Effect of HCl and NaOH on Light Production by MnO_2.

Concentration of HCl.	Light production.	Concentration of NaOH.	Light production.
N/10	Negative.	N/10	Fair.
N/20	"	N/20	Bright.
N/40	"	N/40	Fair.
N/80	Very faint.	N/80	Faint.
N/160	Faint.	N/160	"
Water.	"	Water.	"

SUMMARY.

1. The literature on chemiluminescence of pyrogallol is reviewed and a classification of all known types of chemiluminescent reactions given. A light-producing system consisting of an oxidizer, a peroxide, and an oxidizable substance (pyrogallol) is considered in detail.

2. In addition to substances already recorded, chromates, bichromates, hypochlorites, hypobromites, hypoiodites, chromium and iron salts, colloidal silver, platinum, and oxides of manganese, bloods containing hemocyanin or chlorocruorin, and precipitates of $Fe_2Fe(CN)_6$, $Mn(OH)_2 + Mn(OH)_3$, MnO_2, Mn_2O_3, Ag_2O, and metallic silver and platinum black all give light with pyrogallol and H_2O_2. Many other substances recorded gave no light.

3. Ferrocyanide solutions only give a bright light with pyrogallol + H_2O_2 if first exposed to sunlight and air; ferricyanides give

a faint light only, if exposed to sunlight in presence or absence of air.

4. Perborates, persulfates, Na_2O_2, and BaO_2 can take the place of H_2O_2 with some oxidizers but not with all.

5. No light has ever been observed during the liberation of nascent electrolytic oxygen at anodes of platinum black, manganese dioxide, or lead peroxide, in electrolytes containing pyrogallol.

6. Pyrogallol gives light in absence of peroxide, only with sodium hypochlorite and hypobromite, acid (but not neutral or alkaline) permanganate, and ozone. No light has been observed in presence of alkali (with or without H_2O_2) although rapid oxidation takes place.

7. There is a maximum, minimum, and optimum concentration of pyrogallol and also of potassium ferrocyanide for light production. Above the maximum and below the minimum no light appears. No maximum was found for H_2O_2, up to a concentration of 7.5 per cent.

8. Increase of temperature increases the brightness of the light for each concentration of pyrogallol and of potassium ferrocyanide up to an optimum; then a decrease occurs. The greater the concentration of pyrogallol or of ferrocyanide, the higher the temperature necessary to give light. Increase of temperature decreases the duration of the light.

9. Pyrogallol can be oxidized with light production in fairly strong alcohol, acetone, and ether.

10. Acid prevents and alkali favors the luminescent oxidation of pyrogallol by $MnO_2 + H_2O_2$.

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THE STRUCTURE OF THE PURINE MONONUCLEOTIDES.

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(Received for publication, June 12, 1917.)

A careful examination of the literature on the structure of plant nucleic acid will show that excellent evidence has been furnished for the following conclusions.

1. Nucleic acid is a tetranucleotide; that is to say, it is composed of the groups of four mononucleotides.

2. The four mononucleotide groups of yeast nucleic acid are joined to one another through their carbohydrate groups, giving rise to a polysaccharide structure. The order of the mononucleotide groups in nucleic acid has been determined.

These two deductions are based principally upon the preparation and properties of various simpler nucleotides from yeast nucleic acid,[1] and upon a comparative study of the rate at which phosphoric acid is liberated from them by mild acid hydrolysis.[2]

3. Each mononucleotide is composed of three groups, because by complete acid hydrolysis each produces three substances, ribose, phosphoric acid, and a nitrogenous compound. This has been found in the case of one of the four mononucleotides (guanine mononucleotide) by direct experiment with the substance itself but its extension to the other three mononucleotides, although indirect, is practically conclusive.

[1] Guanine-cytosine dinucleotide, Jones, W., and Richards, A. E., *J. Biol. Chem.*, 1915, xx, 25.

Adenine-uracil dinucleotide, Jones, W., and Read, B. E., *ibid.*, 1917, xxix, 111.

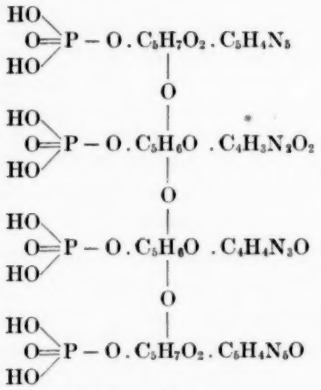
Uracil-cytosine dinucleotide, Jones and Read, *ibid.*, 1917, xxxi, 39.

Guanine mononucleotide, Read, *ibid.*, 1917, xxxi, 47.

[2] Jones and Read, *J. Biol. Chem.*, 1917, xxix, 123.

4. The preparation of four nucleosides from plant nucleic acid proves that in each of the four mononucleotides the nitrogenous group is combined to a ribose group.[3]

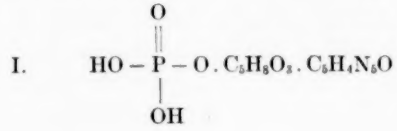
These statements would deal completely with the *gross* structure of nucleic acid as represented in the formula below, if the positions of the phosphoric acid groups in the four mononucleotides were definitely known.

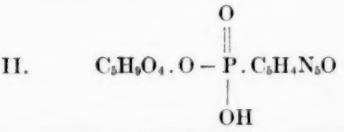


Yeast nucleic acid.

We propose to show the position of the phosphoric acid group in each of the purine mononucleotides.

The three possible arrangements of the three groups in guanine mononucleotide are indicated in the following formulas.





[3] Guanosine, Levene, P. A., and Jacobs, W. A., *Ber. chem. Ges.*, 1909, xlii, 2474; adenosine, 2703; cytidine, *ibid.*, 1910, xliii, 3150.

Uridine, Levene, P. A., and La Forge, F. B., *Ber. chem. Ges.*, 1912, xlv, 608. Read, B. E., and Tottingham, W. E., *J. Biol. Chem.*, 1917, xxxi, 295.

III. $C_5H_9O_4 . C_5H_3N_5O . \begin{matrix} O \\ \| \\ P - OH \\ | \\ OH \end{matrix}$

Guanine mononucleotide forms guanosine, so that its guanine and ribose groups must be directly combined. Moreover, the nucleotide is a dibasic acid that forms a crystalline dibrucine salt. Formula II is therefore excluded.

A crucial experiment described below decides between Formulas I and III. Guanine mononucleotide liberates guanine with much greater rapidity than it liberates phosphoric acid. Formula III is therefore excluded and Formula I alone remains.

Similar reasoning would show that adenine mononucleotide has an analogous structure. But adenine mononucleotide has never been prepared. We have found, however, that adenine-uracil dinucleotide, which can be easily obtained, liberates its adenine far more rapidly than it liberates its phosphoric acid. As the two phosphoric acid groups of the dinucleotide are not directly joined to one another the results obtained may be safely applied to hypothetical adenine mononucleotide.

Guanine Mononucleotide.

Pure guanine mononucleotide was prepared from yeast nucleic acid and allowed to dry in a desiccator with sulfuric acid. The product still contained about 7 per cent of moisture but its state of dryness and even its condition of purity are not essential to the experiments described, as we shall deal with ratios.

Six portions from the same specimen of the nucleotide were weighed into small flasks provided with condensing tubes and each portion was treated with twenty parts of 5 per cent sulfuric acid. The flasks were then submerged in a boiling water bath and heated for various periods from 5 minutes to 3 hours. Each hydrolyzed product was made alkaline with ammonia while still hot, for the precipitation of free guanine, and after standing over night the guanine was filtered off, allowed to dry, and weighed. The filtrates from guanine were heated to the boiling point and treated with magnesia mixture for the precipitation of free phosphoric acid. After standing over night the crystalline

precipitates of magnesium ammonium phosphate were filtered off, allowed to dry, and weighed.

In Table I the quantities of the constituents determined are all calculated for 1 gm. of mononucleotide and the maximum amount obtained in any experiment is assumed to be 100 per cent. From this amount the corresponding percentages for the other experiments are reckoned.

The difference in the rates at which guanine and phosphoric acid are liberated from the nucleotide is so great that it can be attributed neither to imperfections of method nor to analytical error.

TABLE I.

Nucleotide used.	Time of hydrolysis.	Magnesium ammonium phosphate.			Guanine.		
		Obtained.	Per gm. of nucleotide.	Per cent of total.	Obtained.	Per gm. of nucleotide.	Per cent of total.
0.4234	5 min.	0.0499	0.1178	18.8	0.0835	0.1972	50.0
0.5030	15 "	0.1348	0.2680	42.8	0.1747	0.3473	88.1
0.5000	30 "	0.2004	0.4008	64.0	0.1901	0.3802	96.3
0.3487	1 hr.	0.1881	0.5395	86.2	0.1396	0.4002	101.5
0.3360	2 "	0.2017	0.6000	95.8	0.1310	0.3899	98.9
0.3448	3 "	0.2158	0.6259	100.0	0.1360	0.3944	100.0

The results given in Table I are represented diagrammatically in Fig. 1. Its method of construction is so obvious as to require no explanation.

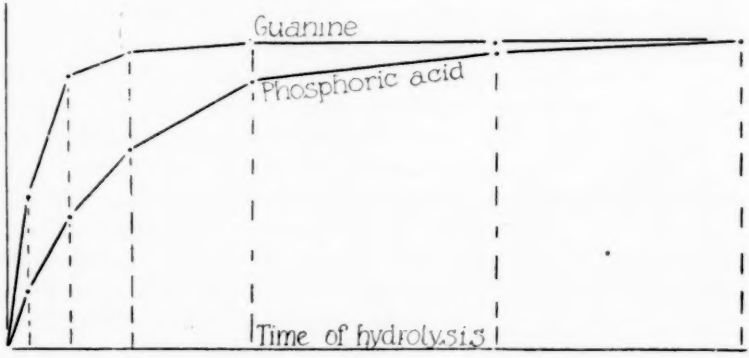


FIG. 1.

Adenine-Uracil Dinucleotide.

Portions of the dinucleotide were heated with dilute sulfuric acid for various periods of time. As guanine was not to be taken into consideration the fluids were made alkaline with ammonia and the free phosphoric acid was determined by precipitating directly with magnesia mixture as described above.

In a second series of experiments with the same specimen of dinucleotide, the hydrolyzed product was made alkaline with ammonia and the adenine was precipitated with an ammoniacal solution of silver nitrate. The gelatinous precipitates of silver-adenine were washed until the wash water contained no trace of either free or combined ammonia, when the filter papers with their precipitates were placed in Kjeldahl flasks and the nitrogen was determined. For each experiment 10 cc. of sulfuric acid, 5 gm. of potassium sulfate, and a few drops of aqueous copper sulfate were used.

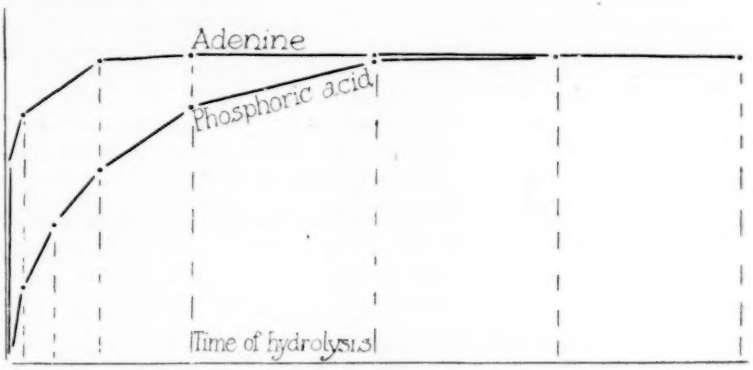


FIG. 2.

The results are given in Tables II and III and are represented diagrammatically in Fig. 2. The methods of tabulation and construction are the same as those used above for guanine mononucleotide with one exception. In the case of the dinucleotide a correction has been made for the small amount of phosphoric acid which is liberated from the pyrimidine nucleotide group. Repeated experiment has shown that this corresponds to 10 mg. of magnesium ammonium phosphate per gm.-hour.[4]

[4] Jones, W., *J. Biol. Chem.*, 1916, xxiv, p. iii. Jones and Read, *ibid.*, 1917, xxix, 111, 123.

TABLE II.

Nucleotide used.	Time of hydrolysis.	Magnesium ammonium phosphate.				
		Obtained.	Per gm. of nucleotide.	Pyrimidine correction.	From purine nucleotide.	Per cent of total.
0.7665	5 min.	0.0726	0.095	0.001	0.094	25.3
0.9712	15 "	0.1638	0.169	0.002	0.167	44.9
0.9754	30 "	0.2330	0.239	0.005	0.234	62.9
0.8832	1 hr.	0.2815	0.319	0.010	0.309	83.1
0.8258	2 hrs.	0.3192	0.387	0.020	0.367	98.7
0.9500	3 "	0.3816	0.402	0.030	0.372	100.0
0.9976	4 "	0.4067	0.408	0.040	0.368	98.9

TABLE III.

Nucleotide used.	Time of hydrolysis.	Adenine nitrogen.		
		Obtained.	Per gm. of nucleotide.	Per cent of total.
0.5245	1 min.	0.0313	0.0597	69.1
0.5592	5 "	0.0393	0.0703	81.4
0.6016	30 "	0.0513	0.0853	98.8
0.5009	1 hr.	0.0436	0.0870	100.8
0.5756	2 "	0.0496	0.0861	99.7
0.5338	3 "	0.0461	0.0864	100.0

THE SIMILARITY OF THE ACTION OF SALTS UPON THE SWELLING OF ANIMAL MEMBRANES AND OF POWDERED COLLOIDS.

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(Received for publication, June 25, 1917.)

I.

1. When dry animal membranes, like pig's bladder freed from fat by prolonged treatment with hot ether (a week or 10 days in a Soxhlet apparatus), are put into water, they will swell; they will also swell and even slightly more when put into a salt solution; but when the membrane is put first for about ½ hour into M/8 solution of NaCl and subsequently into distilled water, the swelling will be twice or three times as great as in either of the other two cases. A further investigation of this problem suggested a possible connection with the structure of the membrane. An investigation of this phenomenon seemed desirable in view of the fact that Flusin[1] has tried to connect the phenomena of osmosis with the imbibition of the separating membrane.

A series of pieces of dry pig's bladder, weighing about 0.4 gm. each, were put into 50 cc. of M/8 NaCl, and left there for half an hour. One was put as a control into distilled water. After a half an hour each piece was freed from adhering solution by putting it between two sheets of filter paper and pressing gently; it was then weighed. The pieces which had been in M/8 NaCl were then put into NaCl solutions of different concentrations, namely, M/8, M/32, M/128, M/512, M/1024, M/4096, M/8192 NaCl, and into H_2O, and the control in H_2O was again put into H_2O. After different intervals the membranes were taken out of the solutions, freed from liquid adhering to their surface, and weighed. Table I gives the results of one such experiment.

[1] Flusin, *Ann. Chim. et Phys.*, 1908, series 8, xiii, 480.

TABLE I.

After	Swelling of dried pig's bladder in per cent of original weight of piece of bladder.								
	In NaCl								Control H_2O
	M/8	M/8	M/8	M/8	M/8	M/8	M/8	M/8	
hrs.									
½	187	186	190	191	184	185	173	183	149
	Then transferred into NaCl								
	M/8	M/32	M/128	M/512	M/1024	M/4096	M/8192	H_2O	H_2O
1½	204	219	289	329	310	328	301	354	165
16½	219	234	363	409	408	455	438	500	187
24	240	242	396	464	451	510	542	562	208

These figures show the following facts: A dried membrane kept permanently in H_2O increases in weight 208 per cent in 24 hours, and when kept permanently in M/8 NaCl it increases in 24 hours slightly more, namely, 240 per cent. This was to be expected on the basis of the old experiments of Hofmeister. What the writer did not expect is the fact that if a piece of membrane is put first for 30 minutes into M/8 NaCl and subsequently for 24 hours into a weaker NaCl solution or distilled water, it swells the more the lower the concentration. Thus a membrane kept for 30 minutes in M/8 NaCl increased its weight when put subsequently into H_2O 562 per cent within 24 hours. The fact that a membrane kept permanently in M/8 NaCl swells so much less (240 per cent) must then be due to the fact that presence of the NaCl solution counteracts the swelling. We must, therefore, discriminate between two effects of the salt solution upon the swelling of a membrane, namely, *first*, a chemical reaction between the membrane and the salt, which would cause in itself a considerable swelling if it were not inhibited by the *second* effect of the solution which counteracts this tendency to swell, and the more so the higher the concentration of the salt solution. We can eliminate this second factor by allowing the membrane to react with the salt first and then transferring the membrane to H_2O after having washed off the adherent salt solution. In a recent series of papers the writer[2] has pointed out the different behavior of

[2] Loeb, J., *J. Biol. Chem.*, 1916, xxvii, 339, 353, 363; 1916–17, xxviii, 175.

the membrane of the eggs of *Fundulus* when taken directly from a salt solution or when taken from distilled water or a solution of a non-electrolyte.

2. When an attempt was made to repeat the experiment just described on pig's bladder with solid blocks of gelatine, either dry or containing varying quantities of water, they failed completely. Gelatine shows none of the peculiarities mentioned in Table I.

A 40 per cent gelatine solution was prepared and after it had set, small pieces were cut out of the jelly and exposed to air for 21 hours during which time they lost about 50 per cent in weight. Then the same experiment as represented in Table I was repeated. Table II gives the result.

TABLE II.

After	Increase in weight of blocks of gelatine in per cent of original weight.								
	In NaCl								Control H_2O
	M/8	M/8	M/8	M/8	M/8	M/8	M/8	M/8	
hrs.									
½	23	24	27	27	25	25	30	24	19.5
	Then transferred into NaCl								
	M/8	M/32	M/128	M/512	M/1024	M/4096	M/8192	H_2O	H_2O
1½	51	52	56	54	51	52	58	50	44
19	198	193	196	190	176	176	158	170	162
24	246	236	239	231	214	216	208	214	188

The swelling in M/8 NaCl was slightly greater than in H_2O, but the gelatine first treated with M/8 NaCl and then put into H_2O for 24 hours did not swell more than the piece kept permanently in M/8 NaCl.

Thin sheets of gelatine behaved like blocks of the same material.

It was thought that the salt had perhaps not entered sufficiently into the gelatine. To avoid this possibility the gelatine was dissolved in M/8 NaCl instead of in H_2O and after setting the pieces were cut out of the gelatine. When put into NaCl solutions varying from M/8 to M/8192 or into H_2O the result was identical with the one expressed in Table II. Nor were the results different when the gelatine used was completely dried beforehand.

3. It was found that finely powdered gelatine behaved exactly like pig's bladder and behaved differently from solid blocks of gelatine. This was true when the powdered gelatine used was the same as that used for making the solid blocks mentioned in Table II.

Commercial Cooper's powdered gelatine was put through a No. 60 sieve and again through a No. 80 sieve. The grains going through the former but not through the latter sieve served for the experiment. 2 gm. of such gelatine were put into a cylindrical funnel, the bottom of which was covered with a round piece of filter paper. The upper surface of the powdered gelatine in the funnel was also covered with a round piece of filter paper in order to make it possible to pour water or salt solution on the gelatine without stirring up the particles too much. When 25 cc. of distilled water are poured on the gelatine, part of the water runs quickly through but part of the water is retained and the mass of gelatine swells. When the process is repeated, only a slight further swelling takes place, and after this no further swelling takes place no matter how much water filters through the gelatine. When instead of letting water run through the gelatine we let 25 cc. of M/8 NaCl run through, and repeat the process of filtering M/8 NaCl through the gelatine, the latter swells also and even a trifle more than in the H_2O experiment, but the maximal swelling is also soon obtained. If, however, we allow first 25 cc. of M/8 NaCl to run through a mass of powdered gelatine and follow this with consecutive washings by distilled water, the mass will swell considerably more with each consecutive washing than in either of the other cases.

The following experiment is the analogue of the one presented in Table I. Eight cylindrical funnels, each containing 2 gm. of powdered Cooper's gelatine (size of grain between sieves No. 60 and No. 80) were prepared in the way described above, and 25 cc. of M/8 NaCl were percolated through each funnel. The mass of gelatine increased in each cylindrical funnel about 18 to 20 mm. in height. After this 100 cc. of a different solution were sent through each of the eight funnels and the additional swelling was ascertained in each mass at the end of the experiment. These solutions were: M/8, M/32, M/128, M/512, M/1024, M/4096, M/8192 NaCl, and H_2O. The results are given in Table III.

TABLE III.

After	Swelling of powdered gelatine in mm. height of a cylinder containing the powder.								
	In NaCl								Control H_2O
	M/8	M/8	M/8	M/8	M/8	M/8	M/8	M/8	
½ hr.....	19.5	20	20	19	17	18	18.5	20	15
	Then 100 cc. of the following solutions were allowed to run through.								
	NaCl							H_2O	H_2O
	M/8	M/32	M/128	M/512	M/1024	M/4096	M/8192		
Additional swelling....	8(?)	5	5	12	21	31	40	64	14

The increase in swelling is expressed in terms of the height of the cylindrical mass of gelatine.

It is obvious that when powdered gelatine is treated first with M/8 NaCl and subsequently with H_2O it retains much more water than when it is perfused permanently with H_2O or permanently with M/8 NaCl. This can also be explained on the assumption that the phenomenon is due to two different effects, first the reaction between salt and gelatine which increases the swelling, and second the inhibition of the swelling if the gelatine is washed in the salt solution. This inhibition reminds us of the inhibition of acid swelling of gelatine by the presence of salts, though the mechanism may be different in the two cases.

From these experiments it is obvious that pig's bladder and powdered gelatine have a peculiarity in common which we were not able to discover in solid blocks of gelatine, namely, to swell considerably more when a short treatment with M/8 NaCl is followed by a treatment with H_2O, than when the mass is treated exclusively with H_2O or exclusively with salt. The property which powdered gelatine and pig's bladder have in common is that they consist of very small discrete particles, grains in the one and fibers in the other; while the gelatine in a block must be considered as a homogeneous mass or as one enormous particle. It should also be stated that this peculiar behavior of powdered gelatine is probably found in many powdered colloids; thus powdered ovomucoid kindly given to us by Dr. López-Suárez behaved exactly like powdered gelatine.

To make the demonstration complete we should add that the after effect of a previous salt treatment just described is found also if other concentrations of NaCl than M/8 are used, namely, M/4, M/2, M/1, etc., but that the after effect ceases when the NaCl concentration is too low, namely, below M/64 or M/128 NaCl.

It should also be said that if we leave powdered colloids or pig's bladder permanently in a salt solution nothing comparable to this after effect is noticed even if the NaCl solution is very weak. In such cases the maximum swelling is soon reached. Table IV gives the results of such an experiment with powdered gelatine, which covers also the case for pig's bladder. We have

TABLE IV.

	Swelling of powdered gelatine in mm. height of a cylinder.											
	NaCl											H_2O
	2 M	M/1	M/2	M/4	M/8	M/16	M/32	M/64	M/128	M/256	M/512	
After first 25 cc. of solution.....	23	22	21	24	22	20	21	21	20	20	21	19
Additional swelling after further 25 cc. of solution.	11	8	6	4	4	2	2.5	2	2	2	2	2
Additional swelling after third 25 cc. of solution....	0	0	0	0	0	0	0	0	0	0	0	0

mentioned the fact that if only M/8 NaCl solutions are allowed to percolate through powdered gelatine the maximum swelling is soon reached and no further swelling takes place. This is true for all concentrations of NaCl tried.

We only notice a slightly increased effect with the increase of the concentration.

II.

The Effect of Different Ions.

1. The excessive swelling, observed in pig's bladder, in powdered gelatine, or in ovomucoid when a short treatment with NaCl solution of not too low a concentration is followed by a treatment with distilled water, is not confined to NaCl, but is produced by

many if not all neutral salts with a univalent cation; while the neutral salts with bivalent cations have no such effect. Pieces of pig's bladder were put for 30 minutes into M/8 LiCl, NaCl, KCl, $MgCl_2$, $CaCl_2$, and $SrCl_2$. Table V gives the result.

TABLE V.

After	Increase in weight of pig's bladder in per cent of original weight in M/8 solutions of:						
	LiCl	NaCl	KCl	$MgCl_2$	$CaCl_2$	$SrCl_2$	Control H_2O
hrs. ½	181	193	186	225	164	162	139
	They were then transferred to distilled water.						
½	306	335	325	169	138	149	145
4	592	547	454	189	138	161	151
22½	662	598	434	195	134	165	145

Leaving aside minor differences, it is obvious that the previous treatment of the membrane with Li, Na, and K causes a considerable increase in swelling, while this increase is lacking in the case of Mg, Ca, and Sr. The salts of the alkali earth simply prevent the subsequent increase in the swelling caused by NaCl or LiCl. Mg is less active than Ca or Sr, which was to be expected. *It cannot be said that $CaCl_2$ or $SrCl_2$ affect the swelling in the opposite way from that caused by NaCl, since the effect of $CaCl_2$ does not differ much from that of distilled water.*

All salts of Na cause the after effect though the quantity of swelling varies with different anions (Table VI).

TABLE VI.

After	Swelling of pig's bladder in per cent of original weight in solutions of different sodium salts.							
	M/8 NaCl	M/8 $NaNO_3$	M/8 $NaCH_3COO$	M/8 Na_2SO_4	M/16 Na_2SO_4	M/8 Na_2 tartrate	M/16 Na_2 tartrate	Control H_2O
hrs. ½	188	209	157	195	222	172	201	167
	They were then transferred to distilled water.							
½	325	312	290	345	464	336	418	189
5	638	464	505	644	846	644	872	221
22½	758	840	490	659	832	1,071	1,400	223

The experiment shows clearly that a short treatment of the membrane with any sodium salt causes a considerable further swelling in H_2O after the free salt solution is leached out. One fact, however, stands out, namely, that the striking difference between the action of univalent and bivalent cations is in no way repeated among the anions. This is in harmony with the writer's first extensive experiments on antagonistic salt action on *Fundulus* in which he showed that the toxic action of high concentrations of salts with univalent cations could be inhibited by small quantities of salts with a bivalent cation, while no such valency effect could be found for the anions.[3]

2. The same difference in the effect of the salts with univalent and bivalent cations upon the subsequent swelling of pig's bladder in distilled water can be found in powdered gelatine. 2 gm. of powdered gelatine (of grain size 60–80) were put into each of a series of cylindrical funnels, and 25 cc. of M/8 LiCl, NaCl, KCl, $MgCl_2$, $CaCl_2$, $SrCl_2$, and $BaCl_2$ were kept in contact with the gelatine for half an hour and then allowed to run off. Four times in succession 25 cc. of distilled water were then allowed to run through each cylinder. Table VII gives the swelling in mm. height of the cylinder.

TABLE VII.

	Swelling in mm. height of cylinder of powdered gelatine under the influence of different chlorides.							
	M/8 LiCl	M/8 NaCl	M/8 KCl	M/8 $MgCl_2$	M/8 $CaCl_2$	M/8 $SrCl_2$	M/8 $BaCl_2$	Control H_2O
Swelling under influence of 25 cc. salt solution.....	23	25	24	24	24	24.5	24	24
Additional swelling under influence of 100 cc. H_2O (four washings).....	15	33	25	4.5	4	3.5	2.5	5.5

The amount of swelling in the different salt solutions was too small to be discovered. In the after effect, however, the striking difference between the salts with univalent and bivalent cations

[3] Loeb, *Arch. ges. Physiol.*, 1901, lxxxviii, 68; *Am. J. Physiol.*, 1901–02, vi, 411.

(which was also noticeable in the case of the swelling of pig's membrane) shows itself. There is practically no difference in the after effect of $CaCl_2$ upon the swelling of the membrane in distilled water and the swelling caused in distilled water without any previous salt treatment.

3. No difference of this kind in the after effect of a treatment with NaCl and $CaCl_2$ could be discovered in solid blocks or sheets of gelatine.

III.

Antagonistic Salt Action.

1. In 1901[3] the writer showed that the injurious effects which a salt with univalent cation has upon the eggs of *Fundulus*, as soon as the concentration of the salt exceeds a certain concentration, can be inhibited by the addition of a very small quantity of a salt with a bivalent cation; while the addition of a salt with a bivalent anion had no such effect. The writer drew from these facts the conclusion that the antagonistic action of the bivalent cation in this case must be due to an action of the salts upon the state of colloids, but for a long time it was found difficult to imitate such an antagonistic salt action directly on colloids. Recently analogies have been shown to exist by Schryver[4] on gels of cholate solutions, by Clowes for soaps,[5] by Lenk[6] for the swelling of gelatine, and by Fenn[7] for the precipitation of dissolved gelatine by alcohol in the presence of different salts.

A very striking antagonistic salt action can be demonstrated in the case of the after effect of NaCl upon the subsequent swelling of pig's bladder and powdered colloids in distilled water. This swelling is inhibited if a comparatively small quantity of

[4] Schryver, S. B., *Proc. Roy. Soc., Series B*, 1914, lxxxvii, 366; 1916, lxxxix, 176. Schryver, S. B., and Hewlett, N., *ibid.*, 1916, lxxxix, 361.

[5] Clowes, G. H. A., *J. Phys. Chem.*, 1916, xx, 407; *Science*, 1916, xliii, 750.

[6] Lenk, E., *Biochem. Z.*, 1916, lxxiii, 15, 58.

[7] Fenn, W. O., *Proc. Nat. Acad. Sc.*, 1916, ii, 534, 539. In one of the examples mentioned by Fenn, *e.g.*, the precipitation of gelatine in mixtures of solutions of Na_3 citrate and $CaCl_2$ by alcohol, the precipitation depends to a large extent upon the formation of a supersaturated solution of calcium citrate which is precipitated by alcohol even if no gelatine is present.

$CaCl_2$ (or any other salt with bivalent cations) is added to the NaCl.

The experiment consisted in the following. Pieces of dry pig's bladder were put for 30 minutes into the following solutions:

50 cc. M/8 NaCl
50 " M/4 " + 1 cc. M/8 $CaCl_2$ + 49 cc. H_2O
50 " M/4 " + 2 " M/8 " + 48 " "
50 " M/4 " + 4 " M/8 " + 46 " "
50 " M/4 " + 8 " M/8 " + 42 " "
50 " M/4 " + 16 " M/8 " + 34 " "
50 " M/4 " + 32 " M/8 " + 18 " "

As the reader will see, each of these solutions was M/8 in regard to NaCl but contained increasing concentrations of $CaCl_2$. After the membranes had been in the solutions for 30 minutes they were transferred to distilled water. It will be seen that the membrane previously treated with pure NaCl swelled considerably while the ones treated previously with NaCl + $CaCl_2$ swelled the less the more $CaCl_2$ was added.

TABLE VIII.

After	Antagonism between NaCl and $CaCl_2$ on the subsequent swelling of pig's bladder when put into distilled water. Increase in weight in per cent of original weight.							
	Cc. M/8 $CaCl_2$ in 100 cc. M/8 NaCl							Control H_2O
	0	1	2	4	8	16	32	
hrs.								
½	189	173	182	185	157	159	164	143
	The membranes were then all transferred to distilled water.							
3	336	305	339	280	155	155	160	
21	345	345	(430?)	269	137	154	167	145

It is obvious that the addition of 4 cc. of M/8 $CaCl_2$ to 100 cc. of M/8 NaCl already inhibits markedly the subsequent swelling of the membrane in distilled water and that the addition of 8 cc. of M/8 $CaCl_2$ to 100 cc. of M/8 NaCl inhibits this swelling almost completely.

If the reader will look over the first row of figures representing the swelling of the membranes while *in* the salt solutions the an-

tagonistic salt action will be seen to be extremely small if it exists at all.

It should also be pointed out once more that the antagonistic action between NaCl and $CaCl_2$ is not the algebraic mean between a swelling effect of NaCl and a dehydrating effect of $CaCl_2$. Table V has already contradicted such an assumption. In membranes not treated previously with any salt the swelling in H_2O amounted in Table VIII to 145 per cent, while in membranes which had been treated for 30 minutes with 100 cc. of M/8 NaCl + 32 cc. of M/8 $CaCl_2$ and were then transferred to H_2O it amounted to 167 per cent. The addition of $CaCl_2$ served only to prevent the enormous increase in weight which a previous treatment with M/8 NaCl alone induces, namely, in this experiment 345 per cent, but it cannot be said that $CaCl_2$ and NaCl affect the membrane in an opposite sense.

2. The antagonism experiment just described is successful also with powdered gelatine. 2 gm. of powdered gelatine (size of grain 60–80) were put into a series of funnels (as described in the beginning of this paper) and first 25 cc. of M/8 NaCl with different quantities of $CaCl_2$ (as described in the experiment with pig's bladder) were allowed to run through the gelatine.

Subsequently 75 cc. of distilled water were allowed to run through each cylinder. The result was striking, inasmuch as *in* the salt solution the swelling was approximately the same in pure NaCl and in NaCl with $CaCl_2$; but in the subsequent treatment with H_2O the swelling was enormous in the powdered gelatine treated previously with pure M/8 NaCl, while this after effect was prevented when 8 cc. or more of $CaCl_2$ were added to 100 cc. M/8 NaCl (Table IX).

Mg and Sr act similarly to $CaCl_2$.

The same antagonistic effect was observed with powdered ovomucoid.

3. The writer has not been able to find such an antagonistic action in the after effect of a previous treatment of solid blocks of gelatine with mixtures of NaCl and $CaCl_2$. The experiment in Table X was performed with solid pieces of dry gelatine.

While in the salt solution the swelling was greater in both M/8 NaCl and M/8 $CaCl_2$ than in pure distilled water, in the subsequent treatment with distilled water no after effect of the na-

ture of that found in powdered gelatine or in pig's bladder could be discovered.

This difference in behavior between powdered gelatine and gelatine in a solid block cannot be ascribed to a difference in the chemical nature of the gelatine used in both cases since the results

TABLE IX.

	Swelling of powdered gelatine in mm. height of a cylinder.							
	Cc. M/8 $CaCl_2$ in 100 cc. M/8 NaCl							Control H_2O
	0	1	2	4	8	16	32	
After 25 cc. salt solution had been added	22	20	23	22	23	21	21	19
	Then 75 cc. distilled water were allowed to filter through.							
Additional swelling after the 75 cc. distilled water had percolated	30	37	29	20	3.5	3.5	5	3

TABLE X.

After	Increase in weight of solid blocks of dry gelatine in per cent of the original weight of the solution.							
	Cc. M/8 $CaCl_2$ in 100 cc. M/8 NaCl						Controls.	
	0	1	2	4	8	16	100 cc. M/8 $CaCl_2$	H_2O
hrs. ½	101	108	99	106	113	99	110	96
	The pieces of gelatine were then transferred to distilled water.							
3	246	265	243	264	265	241	267	257
23	483	550	469	514	524	500	527	538

were the same when the blocks of gelatine were made by dissolving Cooper's powdered gelatine in warm water and allowing the solution to set. The results were also the same when the solid blocks of gelatine were only half dry at the beginning of the experiment, or when they were exposed to the salt solution longer or less than 30 minutes.

IV.

The Influence of the Size of the Particles of Powdered Gelatine upon the Salt Action.

The fact that the after effect of a treatment with neutral salts with monovalent cation is the same for pig's bladder and for powdered gelatine, while it is different for gelatine in the form of one solid block, suggests that we are dealing with a surface effect of the salt. In order to test this suggestion powdered gelatine of four different sizes of particles was prepared, by sifting Cooper's powdered gelatine through sieves with different openings, namely, with 50, 60, 80, 100, and 120 wires per inch. The first lot contained particles which went through 50, but not through 60 (designated 50–60); the next those going through Sieve 60 but not through No. 80 (designated 60–80), and so on. 2 gm. of each of these particles were put into a funnel as described and first 25 cc. of M/8 NaCl were poured on each lot. In order to insure equal action of the salt the solution was kept in contact with the gelatine for $\frac{1}{2}$ hour before it was allowed to filter. The swelling was measured and then 25 cc. of distilled water were poured on the gelatine and the H_2O was kept in contact with the gelatine for 20 minutes before it was allowed to filter through, and the swelling was measured again. This was followed by again pouring 25 cc. of distilled water into each funnel, keeping the H_2O in contact with the gelatine for 45 minutes, then allowing it to run through, and then again measuring the swelling. Table XI gives the result.

TABLE XI.

	Swelling of powdered gelatine of different grain size. Swelling measured in mm. height of the cylindrical mass of gelatine.			
	Size of particles.			
	50–60	60–80	80–100	100–120
Swelling after 25 cc. M/8 NaCl had been allowed to percolate	23	25	24	23
Additional swelling after first 25 cc. H_2O had been allowed to percolate	3	6	8.5	10
Additional swelling after the second 25 cc. H_2O had been allowed to percolate	19	27	31	38

It is obvious that the smaller the particles the greater the retention of water and the greater the swelling. This would be expected if the after effect of the salt upon the swelling in distilled water were a surface effect; for the total surface of a given mass of gelatine is of course the greater the smaller the size of the particles.

It should be possible to calculate the increase in surface with the decrease of the size of the powdered granules if the phenomenon were not complicated by a second variable which acts in the opposite sense as the size of the granules and which the writer believes to be the process of packing. The "packing" diminishes the free area of the particles.

The excessive swelling of pig's bladder under the influence of a previous treatment of NaCl is therefore a phenomenon which can be repeated in powdered gelatine but not in solid blocks of gelatine, and hence must be due to a difference in the structure of the two groups of systems, the gelatine block being homogeneous with only a comparatively small outer surface while the pig's bladder and the mass of powdered gelatine or ovomucoid consist of small discrete elements with an enormous internal surface.

The writer is inclined to believe that the salts combine with the gelatine and as a consequence modify the chemical affinity of the surface of the discrete particles for water. The result is a greater retention of water after the free salt solution has been replaced by distilled water. The gelatine or ovomucoid salts with univalent cations retain the water in such cases very powerfully, while the colloidal Ca salts do not possess this peculiarity. The mechanism of the swelling of powdered colloids or animal membranes like pig's bladder in distilled water, after a previous treatment with a neutral salt with univalent cation, is different from the mechanism of swelling of a solid block of gelatine under the influence of acid or alkali. The latter case has been explained very elegantly by Procter.[8]

[8] Procter, H. R., *J. Chem. Soc.*, 1914, cv, 313. Procter, H. R., and Wilson, J. A., *ibid.*, 1916, cix, 307.

V.

The Antagonistic Salt Action on the Percolation of Water through Powdered Colloids.

In 1905 the writer[9] suggested that the antagonism between salts with monovalent and bivalent cations was due to the fact that small quantities of bivalent cations prevented the diffusion of the salts with univalent cations through the animal membranes. This idea has since been generally accepted and has received support by the work of many experimenters, especially the brilliant experiments of Osterhout[10] on *Laminaria*. It seemed, therefore, of interest to see in which sense the salts with univalent and bivalent cations influence the rate of diffusion or percolation of water through powdered colloids.

We must keep in mind that the antagonistic salt effects described in this paper differ in an essential point from the antagonistic salt effects on the living organism. The observations on the antagonistic action of salts on living organisms were all made while the living object was *in* the salt solution; and the only exceptions from this rule are the observations on washed eggs.[2] The antagonistic effects described in this paper deal with the behavior of colloids *after* the salt solution has been replaced by distilled water and after all the free salt solution has been washed away.

It is very easy to examine the influence of a salt treatment upon the rate of percolation of liquids through powdered colloids. 2 gm. of powdered gelatine or ovomucoid are put into a cylindrical funnel in the way described at the beginning and 25 cc. of a salt solution carefully poured on top of the mass. This solution runs through very rapidly as long as the particles are not too small. This is followed by pouring 25 cc. of distilled water upon the mass and this is then repeated. The rate of percolation becomes slower with each washing (due possibly to a denser packing of the particles) and after two or three washings with distilled water a definite effect of the previous salt treatment upon the rate of percolation can be discovered. This effect is exactly the reverse of the influence of the salts upon the subsequent swelling in

[9] Loeb, *Arch. ges. Physiol.*, 1905, cvii, 252.

[10] Osterhout, W. J. V., *Plant World*, 1913, xvi, 129; *Proc. Am. Phil. Soc.*, 1916, lv, 533.

distilled water. Thus a treatment of powdered gelatine with M/8 NaCl increases the rate of swelling of the mass in distilled water (after the salt solution is washed off) but it diminishes the rate of percolation of distilled water through the mass. $CaCl_2$ neither favors swelling nor does it retard the rate of percolation; it may accelerate it slightly.

It is thus easy to demonstrate an antagonistic salt action upon the rate of percolation of water through powdered gelatine. The same solutions as in Table VIII were used in the following experiment. 1 gm. of powdered gelatine was put into each funnel and at first the various mixtures of NaCl + $CaCl_2$ solutions (25 cc. in each case) were allowed to run through. This was followed by repeated washings with 25 cc. of distilled water. Table XII gives the cc. of water which percolated from the funnels into a measuring cylinder after 25 cc. of H_2O had been poured on the mass for the third time (third washing).

TABLE XII.

Cc. of H_2O which percolated in	Rate of percolation of 25 cc. of distilled water through powdered gelatine after a previous treatment with the following solutions and two washings with 25 cc. H_2O							
	Cc. M/8 $CaCl_2$ in 100 cc. M/8 NaCl							H_2O
	0	1	2	4	8	16	32	
159 min.	9	10.4	13.6	13.2	19.1	18.4	21.4	22.4
17 hrs.	23	24	26*	24	30*	26.5	25.5	27*

* Some of the water retained in previous washings had filtered through.

The rate of percolation is slowest in the gelatine previously treated with M/8 NaCl; and here the swelling is greatest. This is natural since the swelling as well as the lowering of the rate of percolation have the same cause, namely, the retention of water by the powdered gelatine. The same experiment can be made with powdered ovomucoid.

The writer has not yet tried any experiments on the influence of a previous salt treatment on the diffusion of water through a membrane of pig's bladder, though he intends to do so.

The experiments mentioned here bear a certain resemblance to the observations of soil chemists on the percolation of water through soil previously treated with salts. The writer's attention

was called to this work by Professor Lipman in Berkeley, in whose laboratory the subject has recently been investigated by Mr. Sharp.[11] It seems that many years ago A. Meyer first observed the fact that if soil had been soaked with certain salts it became impermeable for water, after the salt had been leached out. Schlösing and Van Bemmelen showed that the phenomenon was connected with a greater degree of suspensibility of the soil after such a treatment.[12] Soil treated with M/8 NaCl becomes almost impermeable for water after the salt solution is washed out. No measurable swelling of the soil follows when the soil is first treated with M/8 NaCl, then with distilled water until all the salt solution is driven out, and the soil becomes highly impermeable. The impermeability in this case is much greater than in the case of powdered gelatine or ovomucoid and the suspicion is justified that the impermeability of the soil after a treatment with NaCl is at least partly due to a denser packing of the particles. This variable may also be at least partly responsible for the retardation of percolation of water through powdered gelatine after a previous treatment with NaCl.

$CaCl_2$ does not retard the subsequent percolation of water through soil and it is easy to demonstrate the antagonism between NaCl and $CaCl_2$ upon the subsequent rate of percolation of water through soil after the salt is leached out.

10 gm. of finely powdered garden soil were put into each of a series of cylindrical funnels. Then 25 cc. of the antagonistic salt mixtures were poured on the soil and the time measured until 20 cc. of the solution had diffused into a measuring cylinder put under the funnel. Then 25 cc. of H_2O were poured into each funnel and again the time for 20 cc. of liquid to run through the soil was measured, and this was repeated three times. Table XIII gives the results.

It may be possible to make practical use of this action of Ca (which seems to be the same for all bivalent cations) for rendering impermeable soil permeable for water.

The writer does not wish to enter into the cause of this behavior of soil beyond mentioning that if all organic matter of the soil is

[11] Sharp, L. T., *Proc. Nat. Acad. Sc.*, 1915, i, 563; *Univ. Cal. Publ. in Agricult. Sc.*, 1916, i, 291.

[12] Van Bemmelen, J. M., *J. prakt. Chem.*, 1881, xxiii, 388

TABLE XIII.

	Time in minutes for 20 cc. of liquid to run through 10 gm. of soil in a cylindrical funnel.							
	Cc. M/8 $CaCl_2$ in 100 cc. M/8 NaCl.							Control H_2O
	0	1	2	4	8	16	32	
25 cc. salt.....	34	37.5	35	30.5	33	33	34	43
25 " H_2O.....	73.5	66	50	46.5	40	34	33.5	44
25 " "	1,185*	1,185	1,185	1,185		66.5	43	68.5

* *I.e.*, over night.

destroyed by ignition a treatment of such soil with NaCl will no longer call forth the striking inhibition of the percolation of H_2O after the salt is leached out; but that an addition of some finely powdered organic colloid (powdered dry oak leaves, gum tragacanth, powdered gelatine, or ovomucoid) can restore to some extent this effect of a previous washing with NaCl. A mixture of finely powdered marble and powdered colloids acts like a mixture of ignited soil and organic colloids.

SUMMARY OF RESULTS.

1. Dried pig's bladder, freed from fat, when treated for a short time with a solution of a salt with univalent cation swells considerably more when subsequently put into distilled water, than it does if it remains permanently in the same salt solution or when it remains permanently in distilled water without a previous salt treatment.

2. It is assumed that this increased swelling of the membrane in distilled water after a previous treatment with one of the salts with univalent cation is due to an interaction between the salt and a constituent (probably protein) of the membrane; when the bladder remains permanently in the salt solution the latter prevents the swelling which takes place as soon as the salt solution is replaced by H_2O or a very weak salt solution.

3. A treatment of the membrane with salts with a bivalent cation (Mg, Ca, Sr, and Ba) does not induce the excessive swelling when the membrane is subsequently exposed to distilled water. Neither does such a treatment induce a dehydration of

the membrane. Membranes previously treated with salts with a bivalent cation swell when afterwards put into distilled water approximately to the same extent as membranes that have not been treated with any salt.

4. The addition of about 8 cc. of M/8 $CaCl_2$ to 100 cc. of M/8 NaC prevents the after effect which a treatment with a pure M/8 NaCl solution produces. It should be noticed that $CaCl_2$ does not influence swelling in the opposite sense from that of NaCl, but that it renders the after effect of the treatment with NaCl impossible in some other way.

5. It is impossible to repeat these effects of a previous salt treatment upon the subsequent swelling in distilled water with solid blocks of gelatine, or with sheets of gelatine.

6. It is, however, possible to repeat them with powdered gelatine or with powdered water-insoluble ovomucoid (and probably a large number of other powdered colloids).

7. The fact that pig's bladder behaves in regard to these phenomena like powdered colloids but not like solid blocks or sheets of gelatine suggests that the salt effects described in this paper are due to an action upon the surface of colloidal particles (fibers in the case of pig's bladder).

8. This suggestion is supported by the fact that the effect of a previous treatment with M/8 NaCl upon the subsequent swelling of a given mass of powdered gelatine in distilled water is greater when the size of the particles is smaller and hence the total internal surface greater.

9. It follows from all this that the mechanism of the swelling described in this paper is of a different nature from that observed in solid masses of gelatine under the influence of acid or alkali.

10. Observations upon the rate of percolation of water show that the effect of salt upon the subsequent rate of percolation of distilled water through the powdered gelatine varies inversely with the rate of swelling. A previous treatment with M/8 NaCl solution retards the percolation of water through the powdered gelatine, while a previous treatment of the mass with M/8 $CaCl_2$ has no such effect. The addition of a small quantity of $CaCl_2$ to NaCl prevents the subsequent retardation of the rate of percolation of water as it prevents the swelling.

11. It has been known that a treatment of soil with NaCl

renders the soil almost impermeable to water after the salt is leached out. In this case, however, no swelling of the soil seems to take place and the writer is not certain whether the influence of a salt treatment upon the percolation of water through powdered gelatine and ovomucoid is identical with or only analogous to that upon the percolation of water through soil.

THE METABOLISM OF SULFUR.

II. THE INFLUENCE OF SMALL AMOUNTS OF CYSTINE ON THE BALANCE OF NITROGEN IN DOGS MAINTAINED ON A LOW PROTEIN DIET.*

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Recent studies of the metabolism of protein with the aid of the more accurate methods of analysis, developed largely by Folin and by Van Slyke and their coworkers, have shown that amino-acids are absorbed as such from the alimentary tract, and circulate in the blood or are stored in the tissues. This has led to a realization of the fact that the rôle of the proteins in nutrition is a function of their component amino-acids, and that the adequacy or inadequacy of any individual protein is dependent upon the quantitative relationships of the amino-acids of its molecule. Attention accordingly has been centered on the problem of the ability of the organism to synthesize or dispense with the various individual amino-acids.

Certain proteins, such as gelatin, have long been known to be lacking in specific amino-acids.

Other proteins not completely lacking in any essential amino-acid may yet contain some of these amino-acids in the molecule in such small amounts as to render a high protein intake necessary to supply a sufficient quantity of the requisite amino-acids. This point of view has been clearly defined by Osborne and Mendel (1) in their studies on the growth requirements of the white rat. These observers have shown that growth on diets containing different proteins in approximately the same percentage relation may vary considerably, provided the percentage of protein in the diet is near the minimum requirement for these animals. On a diet containing a higher

* I wish to express my indebtedness to Professor A. E. Taylor of the University of Pennsylvania for courtesies which have facilitated the continuance of this research at the University of Illinois.

percentage of protein, these variations in nutritive efficiency are less marked or disappear altogether. They have also for certain proteins demonstrated the specific amino-acids the quantity of which determines the minimum for that protein. Thus rats failed to grow at a normal rate on diets containing less than 15 per cent of casein, while the addition of cystine to food containing 9 per cent of casein without further change at once rendered the diet decidedly more adequate for growth. Similar results were obtained with edestin to which lysine was added, and later (2) with lysine and gliadin. Recently by similar methods of study Hogan (3) has shown that for the proteins of the corn kernel, tryptophane is the first limiting factor and lysine the second.

Of the amino-acids, tyrosine (or phenylalanine (4)), tryptophane, lysine, and cystine are generally recognized as essential amino-acids which must be present preformed in adequate amounts in the diet. Ackroyd and Hopkins (5) have recently reported experiments which indicate that either histidine or arginine but not both must be present in the diet for normal nutrition. The claim of the indispensability of cystine preformed in the diet is based largely on indirect evidence or analogy, rather than on direct experimental evidence. With the exception of the work of Osborne and Mendel, in which the addition of cystine to casein, a protein notably low in cystine, was demonstrated to lower the percentage of casein required for normal growth, little experimental work on this point is available. The high cystine content of the proteins of the epithelial tissue, the constant loss of this sulfur-rich protein through the hair, skin, etc., especially in the lower mammals, and the inability to use inorganic sulfates for protein synthesis and growth have led to a belief in the indispensability of preformed cystine in the diet. Practical feeding experiments with swine (6) have also furnished evidence of a possible rôle of cystine. The offspring of sows fed during pregnancy with blood albumin (a protein rich in sulfur and in which a large part of the sulfur is presumably present as cystine) were larger, with heavier and darker coats of hair than the controls. McCollum and Davis (7) have suggested that the loss of sulfur from casein when heated may be associated with the decreased efficiency of such casein as a foodstuff. Holt in a recent address (8) has made the suggestion that the success attained in infant nutrition with whole milk from cows may be due not to the higher percentage of protein as such in whole milk, but to the fact that by thus increasing the percentage of protein in the diet, the infant's actual amino-acid needs for growth, especially in lysine and cystine, may be more nearly satisfied. Abderhalden (9) attempted without success to remove the cystine from completely hydrolyzed protein by precipitation with glacial acetic acid in order to determine the "*biologische Wertigkeit*" of cystine by feeding the resultant product to animals. In acceptance of this belief in the indipensability of cystine, many workers have added cystine to the products of hydrolysis of proteins before feeding, to replace the cystine destroyed in the course of the hydrolysis (Abderhalden (4) and Totani (4), Geiling (5)).

The experiments reported in the present communication represent an attempt to approach the problem of the cystine (and sulfur) requirement of the organism of the dog from the standpoint of the protein minimum, making use of variations in the nitrogen balances as indications of any change in the adequacy of the diet.

The general plan of the experiments has been as follows. Dogs were maintained on standard diets of low protein content but of ample calorific value. After control periods on these diets, small amounts of cystine (0.5 to 1.0 gm. of cystine daily) were added to the standard diets, and the influence of the cystine was noted as evidenced by changes in the nitrogen balances. In certain experiments as additional controls, nitrogen in the form of glycocoll, a dispensable amino-acid, and of tyrosine, an essential amino-acid, was added to the standard diet in amount equivalent to that added in the form of cystine. The animals used were females, and were accustomed to laboratory conditions, one of the animals (Dog A) having been in the writer's possession for over 3 years, the subject of frequent nutrition experiments. The animals were kept in the usual metabolism cages and the urine was separated into 24 hour periods by daily catheterization. There was no evidence of cystitis at any time. Separation of the feces into periods was accomplished by the use of carmine. In order to give consistency and bulk to the feces, neutral calcium phosphate (Merck's "Blue Label") was added to the diet, the pure calcium phosphate being chosen rather than the usual bone ash in order to avoid as far as possible the presence of sulfur in the diet other than the sulfur of the protein. The source of the protein of the diet was beef heart, trimmed from the adjacent fat, finely ground, and carefully mixed to ensure uniformity. In one experiment (Dog G), it was necessary to substitute for the beef heart, finely chopped steak from which connective tissue and fat had been removed as far as possible before grinding. The cane sugar, lard, and starch were pure commercial preparations. The purity of the cystine, which was prepared from wool by acid hydrolysis according to Folin, was established by analyses for nitrogen and sulfur.

Nitrogen was determined by the Kjeldahl-Gunning method. For the determination of sulfur in the food a weighed sample was evaporated to dryness in a porcelain evaporating dish with concentrated nitric acid on the water bath, the dried residue treated with Benedict's copper nitrate-potassium chlorate oxidation mixture, evaporated, and treated as in the method for urine.

The standard daily diets are shown in Table I. In the experiments with Dog A, Series I, and Dogs C and G, the cystine was added without alteration of the diet. In the experiments with Dog A, Series II, and Dog B, in order to keep the nitrogen intake constant, when the amino-acids were fed, an equivalent amount of nitrogen in the form of beef heart was removed from the diet. The starch was first made into a smooth paste with water, the other ingredients were added, and the whole was thoroughly mixed.

TABLE I.

Standard Diets.

	Dog A.		Dog B.	Dog C.	Dog G.
	Series I.	Series II.			
Sucrose, *gm.*	100	50	90	90	70
Starch, *gm.*	40	30	20	40	40
Lard, *gm.*	50	40	50	50	50
Calcium phosphate, *gm.*	5	5	5	5	10
Water, *cc.*	400	400	450	450	500
Total calories*	1,010	680	890	970	890
Calories per kg. body weight**	61	42	74	74	59

* This figure does not include the calories received in the form of beef heart. These varied slightly in individual periods, but approximated 100 calories in all but the experiments with Dog A, Series I, in which the calorific value approximated 125 calories.

** Exclusive of the calories from the beef heart.

The main difficulty experienced in the experiments was a refusal of the food after the experimental diet had been fed for some days. The diets contained much non-nitrogenous material and little meat, so that the mixed food had little to offer in the way of palatability. Although by forced feeding it would no doubt have been possible to prolong the experiments, we have felt that the lack of appetite which would make this necessary, and the inability by forced feeding to administer the food absolutely quantitatively, would render the results of little value. For this reason the two experiments with Dog A were most successful. During two periods of 35 and 42 days respectively, this animal consumed the food completely within 30 minutes after feeding. Dog C refused the diet at the end of 18 days and vomited the food after forced feeding, necessitating a termination of the experiment. Dog G took the diet well up to the 16th day. Forced feeding of a part of the food became necessary on this and on the succeeding day. For this reason the experiment was discontinued.

The details of the experiments are shown in Tables IV to VIII. In every case the addition of small amounts of cystine (equivalent to 0.058 to 0.116 gm. of N) to the low protein diet favorably influenced the nitrogen balance, while the addition of equivalent amounts of nitrogen in the form of glycocoll (Tables VI and VIII) exerted no such influence. The results obtained with tyrosine and phenylalanine (Tables VI and VII) indicate that under the conditions of the experiment these substances had little if any influence on the state of nitrogenous equilibrium, at least no effect

in any way comparable with that of cystine. Thus in Table VI, the addition of 0.75 gm. of cystine to the diet lowered the negative balance from −7.54 gm. for a 6 day period to −2.98 gm., a change from a daily average of −1.26 to −0.50 gm. On the withdrawal of the cystine, the negative balance increased to −5.49 gm. or −0.92 gm. daily, and remained practically constant at −5.64 and −5.44 gm. after the addition of glycocoll and tyrosine in the two following periods. In the next period addition of cystine

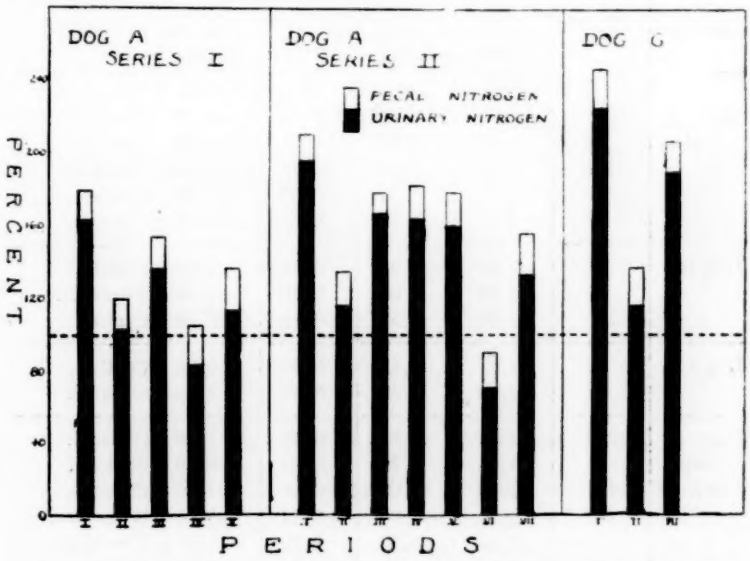

FIG. 1. The nitrogen eliminations are expressed in terms of the percentage of the dietary nitrogen (100 per cent, dotted line). The percentages are computed from the figures in Table II, which should be consulted for an explanation of the diet of the various periods.

resulted in a *positive* nitrogen balance of +0.45 gm. for the period, or +0.07 gm. daily, followed by a negative balance of −3.28 gm. in the subsequent period on removal of the cystine again from the diet. If the 1st day of each period, the transitional day, be omitted from the averages, the changes in the balances become more striking. Table II represents the average daily nitrogen eliminations and balances of the various experiments with the 1st day of each period omitted from the average. From the data in this

table, the excretion of urinary and fecal nitrogen and the nitrogen balances have been computed in terms of the percentage of dietary nitrogen, and the results plotted (Fig. 1) for three of the experiments. The dotted line represents 100 per cent or the nitrogen intake. All that portion of the chart above the dotted line represents the negative balance in terms of the percentage of the intake of nitrogen.

TABLE II.

Animal.	Experiment No.	Nitrogen.				Period.
		Diet.	Urine.	Feces.	Balance.	
		gm.	gm.	gm.	gm.	
Dog A, Series I.	I	1.48	2.41	0.23	−1.16	Control.
	II	1.57	**1.62**	0.26	**−0.31**	**Cystine.**
	III	1.55	2.12	0.27	−0.84	Control.
	IV	1.53	**1.29**	0.32	**−0.08**	**Cystine.**
	V	1.39	1.58	0.32	−0.51	Control.
Dog B.	I	1.22	1.97	0.24	−0.99	Control.
	II	1.22	1.69	0.21	−0.68	Tyrosine.
	III	1.22	**1.26**	0.24	**−0.28**	**Cystine.**
Dog C.	I	1.27	**1.73**	0.28	**−0.74**	**Cystine.**
	II	1.08	2.59	0.38	−1.89	Control.
Dog A, Series II.	I	1.14	2.24	0.16	−1.26	Control.
	II	1.14	**1.32**	0.22	**−0.30**	**Cystine.**
	III	1.14	1.90	0.24	−1.00	Control.
	IV	1.14	1.86	0.22	−0.94	Glycocoll.
	V	1.14	1.82	0.21	−0.89	Tyrosine.
	VI	1.14	**0.81**	0.22	**+0.11**	**Cystine.**
	VII	1.14	1.53	0.25	−0.64	Control.
Dog G.	I	1.28	2.88	0.37	−1.97	Control.
	II	1.54	**1.78**	0.32	**−0.56**	**Cystine.**
	III	1.54	2.92	0.26	−1.64	Glycocoll.

The failure of glycocoll to influence the loss of body nitrogen is of importance in view of the results of Abderhalden (4) which show that the addition of glycocoll or alanine to the diet of mice on a non-protein diet causes a diminished loss of nitrogen from the organism. It might be argued that the effect of cystine is due to a similar action, but the fact that glycocoll and tyrosine when fed

under the same experimental conditions failed to produce a like decrease in the loss of nitrogen from the body speaks against this explanation. We believe that we are dealing with a specific demand for cystine in an animal maintained on a low protein diet, that this diet is adequate or nearly so in its nitrogen content, but that it is deficient in cystine, which is the amino-acid that determines the minimum protein requirements in this case. It is worthy of note that the most successful experiments were obtained with long haired animals, Dogs A and G.

TABLE III.

	Beef heart.	Cat muscle.
	per cent	*per cent*
Total protein	15.01	
" " (N × 6.25)	17.56	
" N	2.81	
" protein N	2.39	2.92
" sulfur	0.180	0.213
" protein S	0.110	0.132
Per cent of N as protein	85.0	
" " " S " "	60.1	61.5*
N:S	15.6	
" (protein)	21.72	22.1*

* Calculated from the figures of Lee, Scott, and Colvin (10).

Concerning the nature and amount of the protein sulfur in the diet fed in these experiments, *i.e.*, beef heart, little information is available. As shown in the tables the diet was low in sulfur as well as in nitrogen. Analyses of the beef heart showed a ratio of total nitrogen to sulfur of 14 – 16 : 1, which is similar to that usually accepted as a general figure for pure proteins. However, considerable amounts of both nitrogen and sulfur are present in beef heart as non-protein extractives so that the above ratio does not necessarily represent the true relationship between protein nitrogen and sulfur. We have, therefore, analyzed the beef heart mixture as fed in these experiments in order to ascertain the relationship between the protein nitrogen and sulfur of the diet under consideration. Analyses of this nature are reported by Lee, Scott, and Colvin (10) for certain muscles of the cat, but no similar analyses of beef heart muscle could be found in the litera-

ture. The protein content of the mixture was determined by the method of Janney (11) and the resultant preparation analyzed for nitrogen and sulfur. The analytical results are presented in Table III, with the figures of Lee, Scott, and Colvin included for purposes of comparison. The results obtained show that the percentage of extractive sulfur in the beef heart is greater than is the percentage of extractive nitrogen; that is, the protein sulfur is lower in proportion to the total sulfur than is protein nitrogen in proportion to the total nitrogen. The N:S ratio of the protein is 21.7 as compared with 15.6, the ratio of the whole beef heart muscle. The protein, therefore, fed in the present series of experiments was lower in its sulfur content than the ordinary proteins of the diet. No attempt was made to determine what proportion of this protein sulfur was present as cystine sulfur. The accumulation of further data on the sulfur and cystine content of various muscles is desirable.

SUMMARY.

The addition of small amounts of cystine to the diet of dogs on a low protein diet diminished the loss of nitrogen from the body and favorably influenced the nitrogen balance. This is interpreted to be the result of a specific demand for cystine for metabolic purposes, since tyrosine and glycocoll added to the diet under like conditions of experimentation did not diminish the nitrogen loss or influence the condition of nitrogenous equilibrium.

TABLE IV.

Dog A. Black Long Haired Female.

Period.	Day.	Weight.	Intake.		Urinary nitrogen.	Fecal nitrogen.	Nitrogen balance.	Diet.
			Nitrogen.	Sulfur.				
		kg.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	
	1	16.59	1.48	0.096	2.77	0.23	−1.52	
	2	16.56	1.48	0.096	2.39	0.23	−1.14	Standard diet and
	3	16.55	1.48	0.096	2.43	0.23	−1.18	50 gm. beef heart
I	4	16.54	1.48	0.096	2.39	0.23	−1.14	daily.
	5	16.54	1.48	0.096	2.39	0.23	−1.14	
	6	16.51	1.48	0.096	2.39	0.23	−1.14	
	7	16.54	1.48	0.096	2.44	0.23	−1.19	
Total			10.36	0.672	17.20	1.61	−8.45	
Average			**1.48**	**0.096**	**2.46**	**0.23**	**−1.21**	
	8	16.57	1.57	0.363	2.08	0.26	−0.77	
	9	16.57	1.57	0.363	1.73	0.26	−0.42	As Period I plus 1.0
	10	16.61	1.57	0.363	1.62	0.26	−0.31	gm. cystine.
II	11	16.57	1.57	0.363	1.65	0.26	−0.34	
	12	16.61	1.57	0.363	1.57	0.26	−0.26	
	13	16.72	1.57	0.363	1.52	0.26	−0.21	
	14	16.76	1.57	0.363	1.62	0.26	−0.31	
Total			10.99	2.541	11.79	1.82	−2.62	
Average			**1.57**	**0.363**	**1.68**	**0.26**	**−0.37**	
	15	16.80	1.55	0.096	1.73	0.27	−0.45	
	16	16.75	1.55	0.096	2.09	0.27	−0.81	As Period I.
	17	16.67	1.55	0.096	2.05	0.27	−0.77	
III	18	16.70	1.55	0.096	2.03	0.27	−0.75	
	19	16.70	1.55	0.096	2.05	0.27	−0.77	
	20	16.71	1.55	0.096	2.19	0.27	−0.91	
	21	16.74	1.55	0.096	2.31	0.27	−1.03	
Total			10.85	0.672	14.45	1.89	−5.49	
Average			**1.55**	**0.096**	**2.06**	**0.27**	**−0.78**	
	22	16.74	1.57	0.230	2.03	0.32	−0.78	
	23	16.75	1.57	0.230	1.26	0.32	−0.01	
	24	16.75	1.57	0.230	1.32	0.32	−0.07	As Period I plus 0.5
IV	25	16.80	1.57	0.230	1.25	0.32	−0.00	gm. cystine.
	26	16.79	1.57	0.230	1.30	0.32	−0.05	
	27	16.85	1.44	0.230	1.28	0.32	−0.16	
	28	16.93	1.44	0.230	1.34	0.32	−0.22	
Total			10.73	1.610	9.78	2.24	−1.29	
Average			**1.53**	**0.230**	**1.40**	**0.32**	**−0.18**	

TABLE IV—*Concluded.*

Period.	Day.	Weight.	Intake.		Urinary nitrogen.	Fecal nitrogen.	Nitrogen balance.	Diet.
			Nitrogen.	Sulfur.				
		kg.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	
	29	16.97	1.39	0.096	1.42	0.32	−0.35	
	30	16.92	1.39	0.096	1.54	0.32	−0.47	
	31	16.89	1.39	0.096	1.63	0.32	−0.56	As Period I.
V	32	16.96	1.39	0.096	1.58	0.32	−0.51	
	33	16.92	1.39	0.096	1.61	0.32	−0.54	
	34	16.93	1.39	0.096	1.55	0.32	−0.48	
	35	16.99	1.39	0.096	1.56	0.32	−0.49	
Total			9.73	0.672	10.89	2.24	−3.40	
Average			**1.39**	**0.096**	**1.56**	**0.32**	**−0.49**	

TABLE V.

Dog C. White Short Haired Female.

Period.	Day.	Weight.	Intake.		Urinary nitrogen.	Fecal nitrogen.	Nitrogen balance.	Diet
			Nitrogen.	Sulfur.				
		kg.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	
	1	13.54	1.27	0.211	1.62	0.28	− 0.63	
	2	13.47	1.27	0.211	1.57	0.28	− 0.57	Standard diet, 40 gm. beef heart and 0.5 gm. cystine.
I	3	13.47	1.27	0.211	1.82	0.28	− 0.83	
	4	13.39	1.27	0.211	1.62	0.28	− 0.63	
	5	13.34	1.27	0.211	1.59	0.28	− 0.60	
	6	13.37	1.27	0.211	2.07	0.28	− 1.08	
Total			7.62	1.266	10.29	1.68	− 4.34	
Average			**1.27**	**0.211**	**1.72**	**0.28**	**− 0.72**	
	7	13.22	1.21	0.077	3.18	0.38	− 2.35	
	8	13.01	1.08	0.077	2.52	0.38	− 1.82	
	9	13.02	1.08	0.077	2.64	0.38	− 1.94	Standard diet and 40 gm. beef heart.
II	10	13.06	1.08	0.077	2.71	0.38	− 2.01	
	11	13.04	1.08	0.077	2.39	0.38	− 1.69	
	12	13.01	1.08	0.077	2.68	0.38	− 1.98	
Total			6.61	0.462	6.12	2.28	−11.79	
Average			**1.10**	**0.077**	**2.69**	**0.38**	**− 1.97**	
III	13	13.04	1.14	0.211	1.69			
	14	12.97	1.14	0.211	1.50			As Period I.
Animal refused to eat; experiment discontinued.								

TABLE VI.

Dog A. Weight 15 Kg.

Period.	Day.	Intake.		Urinary nitrogen.	Fecal nitrogen.	Nitrogen balance.	Diet.
		Nitrogen.	Sulfur.				
		gm.	gm.	gm.	gm.	gm.	
I	1	1.14	0.082	2.21	0.16	−1.23	Standard diet and 40 gm. beef heart.
	2	1.14	0.082	2.06	0.16	−1.08	
	3	1.14	0.082	2.20	0.16	−1.22	
	4	1.14	0.082	2.40	0.16	−1.42	
	5	1.14	0.082	2.22	0.16	−1.24	
	6	1.14	0.082	2.33	0.16	−1.35	
Total		6.84	0.492	13.42	0.96	−7.54	
Average		**1.14**	**0.082**	**2.24**	**0.16**	**−1.26**	
II	7	1.14	0.276	1.92	0.22	−1.00	Standard diet, 37 gm. beef heart, and 0.75 gm. cystine.
	8	1.14	0.276	1.46	0.22	−0.54	
	9	1.14	0.276	1.41	0.22	−0.49	
	10	1.14	0.276	1.33	0.22	−0.41	
	11	1.14	0.276	1.21	0.22	−0.29	
	12	1.14	0.276	1.17	0.22	−0.25	
Total		6.84	1.656	8.50	1.32	−2.98	
Average		**1.14**	**0.276**	**1.42**	**0.22**	**−0.50**	
III	13	1.14	0.082	1.38	0.24	−0.48	As Period I.
	14	1.14	0.082	1.85	0.24	−0.95	
	15	1.14	0.082	1.94	0.24	−1.04	
	16	1.14	0.082	1.94	0.24	−1.04	
	17	1.14	0.082	1.88	0.24	−0.98	
	18	1.14	0.082	1.90	0.24	−1.00	
Total		6.84	0.492	10.89	1.44	−5.49	
Average		**1.14**	**0.082**	**1.82**	**0.24**	**−0.92**	
IV	19	1.14	0.076	1.84	0.22	−0.92	Standard diet, 37 gm. beef heart, and 0.47 gm. glycocoll.
	20	1.14	0.076	1.85	0.22	−0.93	
	21	1.14	0.076	1.87	0.22	−0.95	
	22	1.14	0.076	1.87	0.22	−0.95	
	23	1.14	0.076	1.86	0.22	−0.94	
	24	1.14	0.076	1.87	0.22	−0.95	
Total		6.84	0.456	11.16	1.32	−5.64	
Average		**1.14**	**0.076**	**1.86**	**0.22**	**−0.94**	

TABLE VI—*Concluded.*

Period.	Day.	Intake.		Urinary nitrogen.	Fecal nitrogen.	Nitrogen balance.	Diet.
		Nitrogen.	Sulfur.				
		gm.	*gm.*	*gm.*	*gm.*	*gm.*	
V	25	1.14	0.076	1.94	0.21	−1.01	Standard diet, 37 gm. beef heart, and 1.12 gm. tyrosine.
	26	1.14	0.076	1.94	0.21	−1.01	
	27	1.14	0.076	1.91	0.21	−0.98	
	28	1.14	0.076	1.86	0.21	−0.93	
	29	1.14	0.076	1.69	0.21	−0.76	
	30	1.14	0.076	1.68	0.21	−0.75	
Total		6.84	0.456	11.02	1.26	−5.44	
Average		**1.14**	**0.076**	**1.84**	**0.21**	**−0.91**	
VI	31	1.14	0.276	1.03	0.22	−0.11	As Period II.
	32	1.14	0.276	0.81	0.22	+0.11	
	33	1.14	0.276	0.70	0.22	+0.22	
	34	1.14	0.276	0.75	0.22	+0.17	
	35	1.14	0.276	0.83	0.22	+0.09	
	36	1.14	0.276	0.95	0.22	−0.03	
Total		6.84	1.656	5.07	1.32	+0.45	
Average		**1.14**	**0.276**	**0.85**	**0.22**	**+0.07**	
VII	37	1.14	0.082	0.96	0.25	−0.07	As Period I.
	38	1.14	0.082	1.27	0.25	−0.38	
	39	1.14	0.082	1.55	0.25	−0.66	
	40	1.14	0.082	1.59	0.25	−0.70	
	41	1.14	0.082	1.66	0.25	−0.77	
	42	1.14	0.082	1.59	0.25	−0.70	
Total		6.84	0.492	8.62	1.50	−3.38	
Average		**1.14**	**0.082**	**1.44**	**0.25**	**−0.55**	

TABLE VII.

Dog. B. Short Haired White Female Bull Dog.

Period.	Day.	Weight.	Nitrogen intake.	Urinary nitrogen.	Fecal nitrogen.	Nitrogen balance.	Diet.
		kg.	*gm.*	*gm.*	*gm.*	*gm.*	
I	1	12.36	1.22	1.99	0.24	−1.01	43 gm. beef heart and standard diet.
	2	12.29	1.22	1.91	0.24	−0.93	
	3	12.23	1.22	2.11	0.24	−1.13	
	4	12.20	1.22	1.95	0.24	−0.97	
	5	12.21	1.22	1.83	0.24	−0.85	
	6	12.10	1.22	2.05	0.24	−1.07	
Total			7.32	11.84	1.44	−5.96	
Average			**1.22**	**1.97**	**0.24**	**−0.99**	
II	7	12.00	1.22	1.82	0.21	−0.81	40 gm. beef heart plus 0.9 gm. phenylalanine and standard diet.
	8	12.04	1.22	1.39	0.21	−0.38	
	9	12.00	1.22	1.87	0.21	−0.86	
	10	11.89	1.22	1.69	0.21	−0.69	1.0 gm. tyrosine substituted for phenylalanine.
	11	11.91	1.22	1.68	0.21	−0.68	
	12	11.98	1.22	1.80	0.21	−0.80	
Total			7.32	10.25	1.26	−4.22	
Average			**1.22**	**1.71**	**0.21**	**−0.70**	
III	13	11.90	1.22	1.55	0.24	−0.57	40 gm. beef heart, 0.7 gm. cystine, and standard diet.
	14	11.88	1.22	1.30	0.24	−0.32	
	15	11.82	1.22	1.32	0.24	−0.34	
	16	11.88	1.22	1.27	0.24	−0.29	
	17	11.93	1.22	1.10	0.24	−0.12	
	18	11.93	1.22	1.14	0.24	−0.16	
Total			7.32	7.68	1.44	−1.80	
Average			**1.22**	**1.28**	**0.24**	**−0.30**	

TABLE VIII.

Dog G. Long Haired Collie. Weight 15 Kg.

Period.	Day.	Nitrogen intake.	Urinary nitrogen.	Fecal nitrogen.	Nitrogen balance.	Diet.
		gm.	*gm.*	*gm.*	*gm.*	
	1	1.16	3.27	0.37	−2.48	Standard diet plus beef heart 40 gm.
	2	1.16	3.04	0.37	−2.25	
	3	1.16	2.89	0.37	−2.10	
I	4	1.16	2.88	0.37	−2.09	
	5	1.45	2.81	0.37	−1.73	Hamburg steak 43 gm.
	6	1.45	2.79	0.37	−1.71	
Total		7.54	17.68	2.22	−12.36	
Average		**1.26**	**2.95**	**0.37**	**−2.06**	
	7	1.54	2.44	0.32	−1.22	Standard diet, Hamburg steak 43 gm., plus cystine 0.75 gm.
	8	1.54	1.74	0.32	−0.52	
	9	1.54	1.67	0.32	−0.45	
II	10	1.54	1.67	0.32	−0.45	
	11	1.54	1.86	0.32	−0.64	
	12	1.54	1.94	0.32	−0.72	
Total		9.24	11.32	1.92	−4.00	
Average		**1.54**	**1.89**	**0.32**	**−0.67**	
	13	1.54	1.97	0.26	−0.69	Standard diet, Hamburg steak 43 gm., plus glycocoll 0.45 gm.
	14	1.54	2.67	0.26	−1.39	
III	15	1.54	3.11	0.26	−1.83	
	16	1.54	2.92	0.26	−1.64	
	17	1.54	2.96	0.26	−1.68	
Total		7.70	13.63	1.30	−7.23	
Average		**1.54**	**2.73**	**0.26**	**−1.45**	

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NUTRITION INVESTIGATIONS UPON COTTONSEED MEAL.

III. COTTONSEED FLOUR. THE NATURE OF ITS GROWTH-PROMOTING SUBSTANCES, AND A STUDY IN PROTEIN MINIMUM.

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In the first of a series of studies upon the nutritive value of cottonseed flour,[1] we reported:

"Our results indicate that cottonseed meal does not contain sufficient mineral for growth, is not actively toxic, contains efficient protein, and perhaps fat-soluble growth-promoting substances, similar to those of butter fat, but in less adequate quantities."

A second paper[2] further demonstrates the efficiency of the protein of cottonseed flour for the normal growth, development, and reproduction of the albino rat. It is the purpose of this paper to consider the content in cottonseed flour of growth-essential factors other than protein and mineral, and to report the results of studies of the protein minimum of cottonseed flour.

A preceding report showed that when rats received 50 per cent cottonseed flour in a diet containing no additional growth-promoting substances other than those furnished by cottonseed flour, considerable growth was experienced for 135 to 205 days and that when the amount of cottonseed flour was increased to 70 per cent with no additional growth-promoting substances there has been normal growth for 90 days and continuous growth for 165 to 205 days. These results clearly indicate that 50 per cent cottonseed flour furnishes a considerable amount of growth-pro-

[1] Richardson, A. E., and Green, H. S., *J. Biol. Chem.*, 1916, xxv, 307.

[2] Richardson and Green, *J. Biol. Chem.*, 1917, xxx, 243.

moting substances although probably not enough for normal growth, whereas 70 per cent of the flour furnishes an amount sufficient at least for normal growth during 90 days in spite of the fact that this diet is decidedly deficient in mineral content.[3] However, from these experiments we could draw no conclusions as to whether this growth was due to the fat-soluble or water-soluble growth factor, or to both, or deduce anything as to the relative amounts of these two substances.

We reported also that 50 per cent fat-free, ether-extracted flour in a diet made up with lard and starch failed to induce as pronounced growth or maintain animals without complete failure for as long a period as did the unextracted flour, due probably to the absence of the ether-soluble, growth-essential substance. However, animals grew for 145 to 190 days and then maintained weights on the ether-extracted flour for 240 days, which seems to indicate in the light of the results reported in the present paper that there must be present in the ether-extracted flour a considerable amount of the water-soluble growth-promoting factor.

A series of carefully controlled experimental diets designed to throw light on the relative amounts of fat-soluble and water-soluble growth factors in cottonseed flour has been fed for several weeks.

Composition of Diets.

		per cent	*per cent*
A.	Ether extract cottonseed flour	4.35	12
	Casein	18.	18
	Lard	17.65	10
	Mineral Mixture III	5	5
	Starch	30	30
	Lactose	25	25

[3] Ash analysis of cottonseed flour (Golaz).

	per cent
SiO_2	0.14
Cl	None.
SO_3	0.06
P_2O_5	2.57
K_2O	2.01
CaO	0.26
MgO	0.25
Na_2O	None.

B.	Ether extract cottonseed flour	4.35	12
	Lard	17.65	10
	Casein	18	18
	Mineral Mixture III	5	5
	Starch and water extract of cotton-seed flour	20	20
	Starch	35	35
C.	Casein	18	
	Butter fat	12	
	Lard	12	
	Mineral Mixture III	5	
	Starch and water extract of cotton-seed flour	20	
	Starch	33	
D.	Casein	18	
	Lard	24	
	Mineral Mixture III	5	
	Starch	53	

The casein used in these diets is purified according to the method described by McCollum and Davis.[4] The mineral employed in the rations is supplied by Mineral Mixture III, described by the same authors.[5]

Mineral Mixture III.

	per cent
NaCl	12.31
K_2HPO_3	28.08
$CaH_4(PO_4)_2H_2O$	0.74
$MgSO_4$ (anhydrous)	1.56
Mg citrate	5.75
Na " (anhydrous)	3.12
Ca lactate	46.80
Fe "	1.64

In Diet A the water-soluble growth factor is supplied by lactose of the purity of ordinary reagents.[6]

Diets A and B receive the fat-soluble growth factor contained in the ether extract of cottonseed flour. The extract is made by percolating the flour until the ether comes through colorless. The ether is then driven from the extract by evaporation over a hot

[4] McCollum, E. V., and Davis, M., *J. Biol. Chem.*, 1915, xxiii, 233.
[5] McCollum and Davis, *J. Biol. Chem.*, 1915, xxi, 625.
[6] McCollum and Davis, *J. Biol. Chem.*, 1915, xxiii, 181.

water bath at 60°C. These two diets were first made up with 4.35 per cent of the ether extract, an amount equivalent to 50 per cent flour in the diet. After 11 days the amount was increased to 12 per cent to enable a comparison of the relative amounts of fat-soluble growth-promoting substance contained in butter fat and in cottonseed flour fat.

Diets B and C receive the water-soluble growth factor contained in the water extract of cottonseed flour dried on starch. The cottonseed flour is extracted for 1 hour with frequent stirring with ten times its weight of distilled water. The liquid is filtered free from the flour, acidified with dilute acetic acid, and heated to boiling to coagulate any possible soluble protein. It is then filtered, evaporated down at 60°C., and dried on starch over a water bath at 60°C. with the aid of electric fans. The water extract of 800 gm. of cottonseed flour dried on 200 gm. of starch weighs 120 gm., the weight of both the extract and starch being 320 gm. Therefore 1 gm. of the starch plus extract is equivalent to 2.5 gm. of cottonseed flour, and 20 per cent of this substance in the diet is equivalent to 50 per cent cottonseed flour, which is the amount of flour generally employed in our experimental rations previously discussed.

Diet C receives the fat-soluble growth factor from butter fat.

From Chart 1 it may be seen that stock rats growing normally at the age of 36 days when placed upon Diet A supplying the water-soluble growth-promoting substance from lactose, and containing only 4.35 per cent ether extract of cottonseed flour, equivalent to 50 per cent flour in the diet, do not receive sufficient fat-soluble, growth-promoting substance from this amount of ether extract to continue normal growth. When the amount of ether extract is increased to 12 per cent, comparable to the content of butter fat which induces normal growth and well-being of the animal, all other nutritive factors being favorable, these same animals although retarded in growth during 11 days on the diet containing 4.35 per cent ether extract now resume a normal rate of growth.

When animals receive in their diet the fat-soluble accessory of butter fat and the water-soluble accessory of cottonseed flour equivalent to 50 per cent flour in the diet, as in Diet C, it will be seen from Chart 1 that rats grow perfectly normally.

When both the water-soluble and fat-soluble substances are supplied from 20 per cent water extract and 12 per cent ether extract respectively, of cottonseed flour, it will be seen from Chart 1 that rats grow normally although their growth was retarded during 11 days by the insufficient amount of fat-soluble growth factor supplied by only 4.35 per cent ether extract.

Chart I indicates the behavior of animals on a diet deficient in both the water-soluble and fat-soluble growth-promoting substances. When placed on Diet D the growth of these animals is immediately retarded and after 4 weeks, they begin to lose in weight.

These results indicate that 50 per cent cottonseed flour in a diet furnishes sufficient water-soluble accessory for normal growth, but does not furnish enough fat-soluble accessory. As compared with 12 per cent butter fat, 12 per cent ether extract of cottonseed flour equivalent to 138 per cent of flour is apparently as efficient in supplying the fat-soluble food accessory.

Earlier studies of cottonseed flour[1, 2] reported the efficiency, both for growth and normal reproduction of the albino rat, of diets in which cottonseed flour furnished the only source of protein. These diets furnished an abundance of protein, 25 per cent, as well as the other essentials to normal growth of the rat. To test still further the efficiency of cottonseed proteins as compared with those proteins of known physiological value, a series of experiments was made to determine the protein minimum of cottonseed flour.

The experimental diets contain all the essentials to growth with varying amounts of protein, 4 to 18 per cent as follows.

		per cent
23.	Cottonseed flour	36
	Protein-free milk	10
	Butter fat	12
	Lard	16
	Starch	26
24.	Cottonseed flour	24
	Protein-free milk	10
	Butter fat	12
	Lard	16
	Starch	38

44.	Cottonseed flour	8
	Butter fat	12
	Lard	15
	Protein-free milk	22
	Starch	43
45.	Cottonseed flour	18
	Butter fat	12
	Protein-free milk	22
	Lard	15
	Starch	33
46.	Cottonseed flour	12
	Butter fat	12
	Lard	15
	Protein-free milk	22
	Starch	37

On a diet in which 18 per cent protein is furnished by 36 per cent cottonseed flour as in Diet 23, rats have grown practically normally and are still alive at the age of 410 days. As may be seen from Chart II, the females have grown larger than the average female, whereas the males have been very slightly under size. Three of the four females on this diet have reproduced, Rat 1027 giving birth to three litters, and Rat 1029 to two litters. Of the six families produced on this diet only six young, Nos. 2060–5, from two families, have survived. At the age of 148 days the one male rat is a little above four-fifths of the average weight at that age, while at the age of 159 days the females are slightly under average weight.

When 12 per cent protein is furnished by 24 per cent cottonseed flour as in Diet 24 the majority of the animals have not grown normally (Chart III) although Female 1028 attained normal full growth quicker than the average individual. Only one of three females ever reproduced, No. 262, giving birth to three females, Nos. 2029, 2030, 2031, all of which are alive at the age of 269 days, fine appearing animals but below average size.

On Diet 45 furnishing only 9 per cent protein from cottonseed flour, all the animals are decidedly under weight at the age of 165 days. But Female 310 at the age of 155 days gave birth to eight fine looking young, all of which she devoured (Chart IV). These results do not altogether agree with the work reported by

Osborne and Mendel.[7] They obtained normal growth with 9 per cent cottonseed protein although they report no reproduction.

Similarly, as seen by Chart IV, on Diet 46 which furnishes only 6 per cent cottonseed protein our rats have been able to show very little growth during 108 days, the animals averaging only 8 to 9 gm. increase in weight, whereas Osborne and Mendel have obtained considerable growth on 6 per cent cottonseed protein.

With Diet 44 containing only 4 per cent protein, an amount smaller than any considered by Osborne and Mendel, rats have behaved as indicated by Chart IV. When first placed on this diet there is a decided loss in weight for several days, after which there is an almost successful attempt at maintenance for about 50 days. These animals are still continued on this diet and though stunted are in good condition, extremely active, and have fine coats of fur.

SUMMARY.

1. 20 per cent of the water extract of cottonseed flour dried on starch, equivalent per gm. to 2.5 gm. of cottonseed flour, *i.e.*, 50 per cent cottonseed flour in the diet, contains sufficient water-soluble food accessory for normal growth.

2. 4.35 per cent of the ether extract of cottonseed flour equivalent per gm. to 11.5 gm. of cottonseed flour, *i.e.*, 50 per cent cottonseed flour in the diet, does not contain sufficient fat-soluble food accessory for normal growth, but 12 per cent of the ether extract appears quite as efficient in supplying enough of the fat-soluble accessory for normal growth as does an equivalent amount of butter fat.

3. 18 per cent cottonseed protein when supplied with adequate amounts of all other necessary nutritive factors induces practically normal growth of the male rat, and better than average growth in the female, and fairly normal reproduction, with high mortality among the second generation. At the age of 148 days the male of the second generation is about four-fifths average size and the female slightly under size.

4. 12 per cent cottonseed protein does not induce perfectly normal growth. On this diet one female has borne three young,

[7] Osborne, T. B., and Mendel, L. B., *J. Biol. Chem.*, 1917, xxix, 289.

all of which are alive at the age of 269 days, although below average size.

5. Normal growth has not been obtained on 9 per cent cottonseed protein but at the age of 155 days one animal, No. 310, has borne a fine looking litter of eight young, all of which she devoured.

6. Very little growth has been obtained with 6 per cent protein, the average gain in weight during 108 days being 8 to 9 gm.

7. With only 4 per cent cottonseed protein rats have fallen off in weight when first placed upon this diet but have almost successfully maintained their weight for 50 days thereafter.

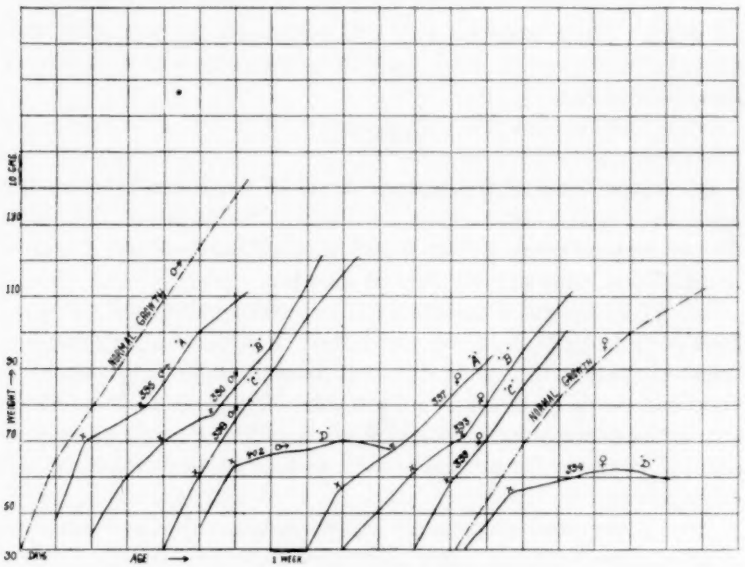

CHART I. X indicates the point at which the rats were given the experimental diets. With animals on diets A and B, XZ indicates the period during which the diet contained 4.35 per cent ether extract of cottonseed flour. Z indicates the point at which the amount of ether extract was increased to 12 per cent. Rats receiving 20 per cent water extract of cottonseed flour with all other nutritive factors favorable in Diet C grow normally. Rats receiving 12 per cent ether extract of cottonseed flour with all the nutritive factors favorable in Diet A, period YZ, grow practically normally. Rats receiving both water and ether extracts of cottonseed flour in Diet B, period Z, grow normally. Rats on Diet D lacking in both water-soluble and fat-soluble food accessories do not continue normal growth.

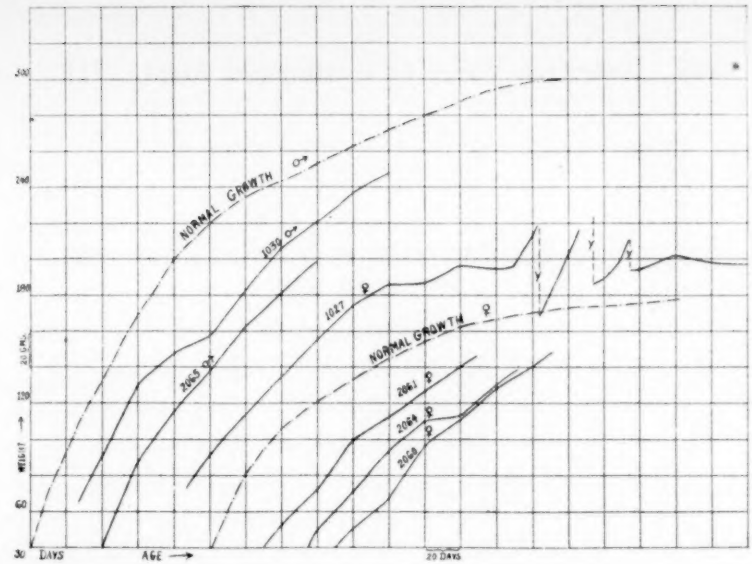

CHART II. Indicates the behavior of animals on Diet 23, furnishing 18 per cent cottonseed protein. Rats 2060, 2061, 2064, and 2065 are of the second generation on this diet raised since weaning on Diet 23.

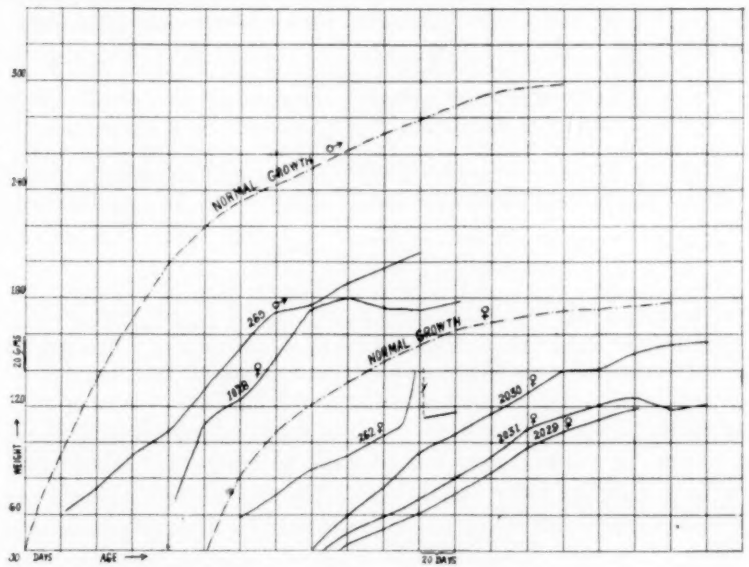

CHART III. Indicates the behavior of animals on Diet 24, furnishing 12 per cent cottonseed protein. Rats 2029, 2030, and 2031 are of the second generation on this diet raised since weaning on Diet 24.

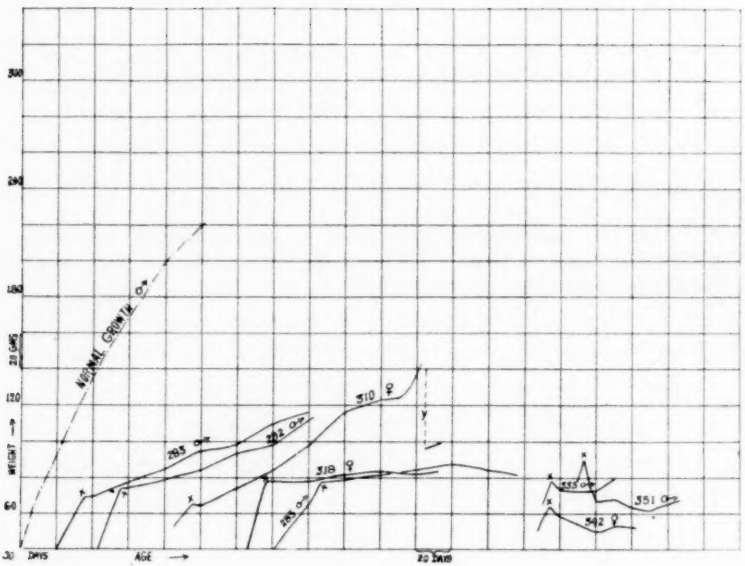

CHART IV. The curves of Rats 283 and 292 indicate the behavior of animals receiving 9 per cent cottonseed protein in Diet 45. Rats 318 and 285 have received 6 per cent cottonseed protein in Diet 46. Rats 335, 342, and 351 received only 4 per cent cottonseed protein in Diet 44.

THE AVAILABILITY OF THE ENERGY OF FOOD FOR GROWTH.*

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Food which enters the animal body has a certain amount of total energy, called the heat of combustion. This energy is different for different feeds. Of this total energy the animal loses part by way of the feces in the undigested food residues, part by way of the urine in incompletely oxidized bodies, and part by way of combustible gases voided. The amount over and above these losses is called the metabolizable energy. Not all of this metabolizable energy is available for the uses of the animal body in either maintenance or growth. There is a loss due to the work of digestion, mastication, and movement of the food through the digestive tract. There is also a further loss due to a stimulated metabolism upon the absorption of digestible substances from the alimentary tract. There may be a slightly greater muscular activity due to the increased food consumed. All this energy is converted into heat and lost from the body. What is left of the metabolizable energy after these second losses are accounted for is called the net, or available, energy. This may be used for production of work, or may be stored in the animal body in the form of protein, fat, or other body substances.

In connection with the general "Use of Food Experiment" conducted at the University of Missouri Agricultural Experiment Station since 1907, some data have been obtained upon the relative amount of the energy of the food which may be recovered in flesh gained. The animals used were mature beef steers, 2 or 3 years old, of the Shorthorn breed. They were as nearly alike in body weight, previous method of treatment, and type as it is possible to select beef steers. One animal was somewhat heavier than the other two. The ration used in the work consisted of five parts of mixed grain to two parts of alfalfa hay. The mixed grain

* Read before the Division of Biological Chemistry of the American Chemical Society, April 12, 1917.

was eight parts maize meal (corn chop) and one part old process linseed meal.

The digestibility of the ration was determined by digestion trials. The cost of maintaining the animals at constant weight was determined by extended maintenance trials. Greater details will be found in previously published work.[1] After the maintenance trial one animal, Steer 18, was slaughtered for analysis. Two other animals were fattened, one to full prime condition, Steer 48, and the other to a condition 40 to 50 days under prime, Steer 121, when they were slaughtered and analyzed (Table I).

TABLE I.

Composition of Animals.

	Weight.		
	Steer 18.	Steer 121.	Steer 48.
Warm empty weight, *gm.*	302,183	508,513	744,708
Per cent of water	57.34	50.02	41.73
Weight of water, *gm.*	173,259	254,339	310,750
Per cent of fat	18.03	29.72	41.25
Weight of fat, *gm.*	54,479	151,131	307,164
Per cent of nitrogen	2.96	2.51	2.07
Weight of nitrogen, *gm.*	8,955	12,776	15,391
Weight of protein, *gm.*	55,968	79,847	96,194
Per cent of ash	5.70	4.14	3.45
Weight of ash, *gm.*	17,211	21,064	25,697
Per cent of phosphorus	1.07	0.74	0.62
Weight of phosphorus, *gm.*	3,230	3,769	4,648

Since Steer 121 during maintenance, or at the beginning of the full fed period, weighed less than the check animal and Steer 48 weighed more it is necessary to calculate their composition assuming the same percentage composition as the check animal had. Table II shows the results together with the calculations for the composition of the gain.

In calculating the thermal equivalent of the fat and protein gained it was necessary to use the data of other investigators. For protein the value of 5.6776 calories per gm. was used. This

[1] Trowbridge, P. F., Moulton, C. R., and Haigh, L. D., *Missouri Agric. Exp. Station Research Bull. 18*, 1915.

is the value found by Köhler[2] for the lean muscular tissue of beef cattle from which the fat had been removed by ether and a correction made for the fat in the residue as determined by the Dormeyer[3] method. For fat the value of 9.4889 calories per gm. was used. This is the average of four results for beef fat quoted by Fries,[4] namely, those of Stohman and Langbein, Stohman and associates, Gibson, and Danilewsky.

Steer 121 stored up 926,359 calories in the fat gained and 141,252 calories in the protein gained. This is a total of 1,067,611 calories. Steer 48 stored up 2,355,601 calories in fat gained and

TABLE II.

Composition of Gain. Energy Stored in Flesh Gained.

	Steer 121.		Steer 48.	
	Flesh gained (estimated).	Composition of gain (estimated).	Flesh gained (estimated).	Composition of gain (estimated).
	gm.	*per cent*	*gm.*	*per cent*
Warm empty weight	211,726	.....	417,900	.....
Water	84,174	39.76	123,372	29.52
Fat	97,626	46.11	248,246	59.40
Nitrogen	3,981	1.88	5,707	1.37
Protein	24,879	11.75	35,667	8.54
Ash	4,161	1.97	7,084	1.70
Phosphorus	597	0.28	1,155	0.28
Energy in fat, *calories*	926,359		2,355,601	
" " protein, "	141,252		202,502	
Total energy stored, "	1,067,611		2,558,103	

202,502 calories in protein gained. This is a total of 2,558,103 calories.

In order to make these gains and store this energy these animals consumed a large amount of feed. Steer 121 consumed over 2,000 pounds of digestible organic nutrients and Steer 48 consumed nearly 8,000 pounds. The equivalent metabolizable energy was found by the method of Armsby.[5] For this ration it is 3,803

[2] Köhler, A., *Z. physiol. Chem.*, 1900–01, xxxi, 479.

[3] Dormeyer, C., *Arch. ges. Physiol.*, 1896–97, lxv, 102.

[4] Fries, J. A., *U. S. Dept. Agric., Bureau Animal Industry, Bull. 94*, 1907, 13.

[5] Armsby, H. P., and Fries, J. A., *J. Agric. Research*, 1914–15, iii, 451.

calories per kg. of digestible organic matter, or 1.72 therms per pound. A therm is 1,000 large calories. Table III gives the data.

The metabolizable energy that may be used for production of flesh is that amount over and above the needs for maintenance. Using the average weight of the animal while on maintenance and the maintenance cost found by trial for each, the cost of maintenance during the full feed period was calculated. The amounts

TABLE III.

Gross and Net Cost of Gain. Per Cent Availability of Energy.

	Steer 121.	Steer 48.
Length of period, *days*	153	567
Weight at beginning, *lbs.*	764	842
" " end, *lbs.*	1,266	1,805
" gained, *lbs.*	502	963
Grain eaten daily, *lbs.*	18.34	16.93
Hay eaten daily, *lbs.*	7.21	7.01
Organic nutrients, *lbs.*	3,398	11,821
Digestible organic nutrients, *lbs.*	2,267	7,860
Metabolizable energy, *therms*	3,900	13,519
Energy per pound gain, *therms*	7.77	14.03
Average weight of animal, *lbs.*	1,041	1,384
Energy per 1,000 pounds for maintenance, *therms*	12.14	12.73
Total energy for maintenance, *therms*	1,900	8,646
Energy above maintenance, *therms*	2,000	4,873
Energy above maintenance per pound of gain, *therms*	3.98	5.06
Energy recovered in gain, *therms*	1,067.6	2,558.1
Metabolizable energy recovered, *per cent.*	53.39	52.49

of energy required for maintenance at different body weights are proportional to the body surfaces, that is, roughly to the two-thirds power of the body weights. The author has shown in previously published work[6] that the surface area of a thin or medium fleshed steer is more nearly proportional to the five-eighths power of the weight, while with very fat steers the five-ninths power should be used. For animals here discussed the five-ninths power was used.

[6] Moulton, C. R., *J. Biol. Chem.*, 1916, xxiv, 299.

According to the theory in the above paragraph the cost of maintenance for the thin animals, Steer 121 and Steer 48, should be proportional to the five-eighths power of the weights until they could be classed as fat and after that the five-ninths power should be used. A calculation was made using the extreme case of the five-eighths power entirely. This made a difference of 0.25 per cent of the net energy cost of a pound of gain for Steer 18 and 1.5 per cent for Steer 48 throughout the entire period. The true value would lie between the one given in Table III and a value smaller by the amount just shown. Therefore the error could hardly be more than one-half that shown, or about 0.75 per cent of the total amount for Steer 48. The error in the calculation of the per cent of available energy would be about double this error. The small size of the error involved makes it inadvisable to use a more complex method of calculation than that employed in the preceding paragraph.

The results of the calculations, given in Table III, show a much higher productive energy cost of each pound of gain for the very fat steer than for the medium fat steer. Table II shows that the gains of Steer 48 were about 29 per cent more fat than those of Steer 121. The productive energy consumed increased in about the same proportion (27 per cent) from 3.98 therms per pound to 5.06 therms.

In the tissue gained by these animals one recovered 53.39 per cent of the metabolizable energy consumed above maintenance and the other recovered 52.49 per cent. Thus it is seen that the very fat steer saved up almost as much of the energy above maintenance as did the medium fat steer. These figures average 52.94 per cent. Since this proportion of the energy is recovered it may be said that this is a measure of the availability and that the metabolizable energy of the ration here used is 52.94 per cent available, or net.

In the work of Armsby, previously referred to, animals were used similar to those here discussed and a ration—alfalfa hay and grain mixture No. 2—somewhat similar was used. Using the data given[7] the writer has calculated the availability. On an average 56.09 per cent of the metabolizable energy was available.

[7] Armsby and Fries,[5] pp. 443, 474.

Since the ration used by Armsby was richer in grain than the one used at the Missouri Agricultural Experiment Station it is advisable to calculate the availability from figures given elsewhere by Armsby. He shows[8] that alfalfa hay has 44 per cent of metabolizable and 17 per cent of net energy. This makes the net to be 38.636 per cent of the metabolizable. For grain mixture No. 2 he shows 65 per cent of metabolizable energy and 40 per cent of net energy. This makes the net energy to be 61.538 per cent of the metabolizable. Putting these two together in the ratio of five parts of grain to two parts of hay (the Missouri ratio) the value of 54.995 per cent is obtained. Armsby's figures show the ration to be 55 per cent available, while the energy stored by the steers used in the work discussed in this paper shows the ration to be about 53 per cent available. This is a remarkably close agreement and is an experimental verification of the work done by Armsby in his calorimeter.

[8] Armsby and Fries, *Penn. State College Agric. Exp. Station Bull. 142*, 1916, 13.

CONTRIBUTIONS TO THE CHEMICAL DIFFERENTIATION OF THE CENTRAL NERVOUS SYSTEM.

IV. THE RELATIVE AMOUNT OF SHEATHING SUBSTANCE IN THE CORPUS CALLOSUM AND INTRADURAL NERVE ROOTS (MAN AND DOG).

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A comparison of the chemical composition of the nerve fibers from the corpus callosum and from the lumbosacral intradural nerve roots in man and in the dog was undertaken in order to determine whether in these two mammals the relative amount of sheathing substance is the same in the two divisions of the nervous system, the callosum representing the central and the nerve roots the peripheral system.

By sheathing substance is meant the myelin sheath which surrounds the axis cylinder and which gives myelinated nerve fibers their glistening white appearance. The term "white matter" has been applied to groups of myelinated fibers as these appear in the

* In 1911–12 this work was begun at the University of Chicago, at the suggestion of my brother, Dr. Waldemar Koch, under a grant from the Wistar Institute of Anatomy and Biology. At this time the dog material was collected and analyzed, also the human corpus callosum, which was obtained through the kindness of Dr. H. Gideon Wells of the Department of Pathology. Dr. Koch died in 1912 and since then I have completed the work. The three samples of human intradural nerve roots were obtained through the kindness of Dr. G. H. Whipple of the Department of Pathology, Johns Hopkins Medical School. Into the period of my present position at the Psychiatric Institute falls the bringing together and working up of the results.

I wish to express my appreciation to Dr. H. H. Donaldson for his interest and aid in bringing these data into shape for publication.

M. L. K.

central nervous system. Barring the insignificant longitudinal striæ and the glia cells, the corpus callosum is purely white matter.

The lumbosacral intradural nerve roots, forming the cauda equina, emerge from the lower levels of the cord and run for some distance within the dura. These also are groups of myelinated fibers.

Both these parts represent white matter as distinguished from gray, but the callosal fibers are without a neurilemma and are intermingled with neuroglia elements, while the intradural nerve roots contain fibers with a neurilemma and are held together by connective tissue, which, nevertheless, is scanty in this locality.

TABLE I.

The Percentage of Water and of Ether-Alcohol Extract Based on the Weight of Dry Substance in the Myelinated Fibers of the Corpus Callosum and of the Lumbosacral Intradural Nerve Roots of the Mature Dog (Hatai).

Dog.	Corpus callosum.		Intradural nerve roots.	
	Water.	Extract.	Water.	Extract.
	per cent	*per cent*	*per cent*	*per cent*
Fat (old)	66.94	70.54	66.44	73.13
Nearly fat-free (old)	69.58	67.90	69.97	68.76
" "	70.21	67.37	70.45	68.36
Fat moderate	71.21	66.71	70.50	66.94
Average	69.58	68.13	69.34	69.29

Hatai[1] found in the dog a great similarity between the water content and the ether-alcohol extract of the corpus callosum on the one hand and of the intradural nerve roots on the other (Table I).

Our own observations on this point by a method like that used by Hatai, show similar volume relations not only in the dog but also in man (Table II) although the values for the intradural nerve roots of man deviate somewhat from those to be expected.

[1] Dr. Hatai's data have not been published heretofore, but he kindly permits us to use them in this paper. It is to be noted that the ether-alcohol extract obtained by Dr. Hatai is not exactly equivalent to the "extract fraction" of the latter tables as the latter includes besides the ether-alcohol extract the water extract.

TABLE II.

he Percentage of Water and of Ether-Alcohol Extract Based on the Weight of ry Substance in the Myelinated Fibers of the Corpus Callosum and of the Lumbosacral Intradural Nerve Roots of the Mature Dog and Man (Koch).

Animal.	Corpus callosum.		Intradural nerve roots.	
	Water.	Extract.	Water.	Extract.
	per cent	*per cent*	*per cent*	*per cent*
g (average of two)	70.11	69.21	69.32	71.46
Man " " "	70.31	70.75	72.18	64.71

These results of Hatai led Donaldson to conclude that the degree of myelination was probably very similar in these two parts of the nervous system. Assuming that the volume of the myelin in the myelinated fibers of the peripheral nerves is about equal to, or in some cases greater than, that of the axis cylinder,[2] then if the general chemical composition was the same it would follow that similar volume relations were true for the central fibers. If we should moreover find that the chemical constituents, especially those predominating in the sheath, were in corresponding proportions, the argument would be fairly complete that the relative amount of sheathing substance is the same or nearly the same in these two types of white matter.

Nothing is definitely known of the function of the myelin sheath although, according to Mathews' Physiological Chemistry (1915), it is probably nutritive. It has a definite chemical composition, however, as distinguished from gray matter (Table III, column C, cortex, and column F, corpus callosum).

The data show that the white matter (corpus callosum) is poorer in water, proteins, and extractives, and richer in lipoids as compared with the gray matter. All the lipoids which were determined, even the phosphatides, are present in greater amounts in the white matter than in the gray matter.

The white matter also undergoes greater changes in its chemical composition than the gray matter during the development of the nervous system (see Table III). Donaldson[3] has shown that during the post-natal development of the mammalian nervous

[2] Donaldson, H. H., and Hoke, G. W., *J. Comp. Neurol. and Psychol.*, 1905, xv, 1. Greenman, M. J., *J. Comp. Neurol.*, 1913, xxiii, 479; 1917, xxvii, 403.

[3] Donaldson, H. H., *J. Comp. Neurol.*, 1916, xxvi, 444.

TABLE III.

*Man. Corpus Callosum and Cortex at Different Ages. Proportions of Constituents in Percentage of Solids.**

	A.	B.	C.	D.	E.	F.
	Whole brain.	Cortex.**	Cortex.**	Corpus callosum.†		
Laboratory No....	13	14	15	W. 41	14	15
Age.....	6 weeks.	2 years.	19 years.	Full term fetus.	2 years.	19 years.
1.						
Water, *per cent*....	88.78	84.49	83.17	89.92	76.45	69.70
Solids, " "	11.22	15.51	16.83	10.08	23.56	30.30
2.						
Proteins.....	46.6	48.4	47.1	48.4	31.9	27.1
Extract.....	53.4	51.6	52.9	51.6	68.1	72.9
Extractives (organic extractives and inorganic constituents).....	20.3	15.8	15.4	18.2	9.1	6.3
Lipoids.....	33.1	35.8	37.5	33.4	59.0	66.5
3.						
Phosphatides.....	24.2	24.7	23.7	23.12	26.3	31.00
Cerebrosides.....	6.9	8.6	8.8	.....	17.2	16.60
Sulfatides.....	1.6	....	1.2	3.33	....‡	8.65
Cholesterol (by difference).....	(0.4)	(2.5)	(3.8)	.....	....	(10.25)

* Koch, W., and Mann, S. A., *J. Physiol.*, 1907, xxxvi, 1. Table III is somewhat modified from the original table to make it comparable with the other tables in the present paper. Data for corpus callosum of full term fetus are added.

** Collection of gray matter; see Koch, W., *Am. J. Physiol.*, 1904, xi, 303–329.

† Collection of white matter; see p. 401

‡ 0.5 as SO_4.

system (rat), the progressive diminution of the water content of the entire brain and of the spinal cord, which is preeminently a function of age, is due mainly to the accumulation of myelin, using the term myelin to designate the sheathing substance, while the cell bodies and their axons do not suffer any significant loss

of water. The white matter loses from 18 to 20 per cent of water, while, according to the admixture of myelinated fibers, the gray matter loses from 2 to 5 per cent of water from birth to maturity. This loss of water is accompanied by changes in the relative proportions of the proteins and extract (including lipoids and extractives). These proportions are changed most in the white matter (corpus callosum), while in the gray matter (cortex) the relative proportions are only slightly altered, the main change being in the relation of the lipoids to the extractives in the extract fraction (Table III).

When we compare the brains of fetal pigs (5 and 10 cm. in length) and the rat at birth—both of which represent gray matter[4] with adult nervous tissue, we find the total lipoid content to be only about one-half as great as at maturity, while certain lipoids, such as the cerebrosides, are entirely absent and the sulfatides are present only in small amount at this stage (Table IV). The phosphatides are present in fairly large amount. The cholesterol was not determined in this series although it is probably present in small amount as an analysis of the brains of fetal dogs indicates (3.76 per cent) (determined directly by the Windaus[5] digitonin method).

TABLE IV.

Lipoids in the Brain of Fetal Pigs and of Albino Rats at Birth, Compared with the Lipoids in the Brain of the Adult Albino Rat (in Percentage of Solids).

	Total lipoids.	Cerebrosides.	Sulfatides.	Phosphatides.
5 cm. fetal pig	21.35	0.00	0.92	15.41
10 " " "	23.43	0.00	0.90	15.62
Rat at birth	24.87	0.00	1.45	15.20
Adult rat	41.70	9.00	4.60	22.00

The great increase of lipoids shown in Table IV, an increase which takes place after about the 10th day of age in the rat, is due for the most part to the formation of the myelin sheaths.

In our chemical studies[6] on the progressive changes in the central nervous system during growth, which were followed in the

[4] Koch, M. L., *J. Biol. Chem.*, 1913, xiv, 267.

[5] Windaus, A., *Z. physiol. Chem.*, 1910, lv, 113.

[6] Koch, W., and Koch, M. L., *J. Biol. Chem.*, 1913, xv, 423.

albino rat, we found that the most marked and characteristic of these chemical changes which occur during and just after the appearance of the myelin sheath are largely noticeable in the proportions of the individual lipoids which appear coincident with myelination. For example the phosphatides—which are not very differently distributed between the cell body and nerve fiber as indicated by a comparison of cortex with callosum[7] and which are present before myelination—increase at a remarkable rate at the beginning of myelination[8] and as this process proceeds, sulfatides, cerebrosides, and cholesterol are elaborated and laid down. These therefore are found chiefly in the myelin sheath.

It was suggested by one of us (W. K.) that the sulfatides (lipoid sulfur), which have been shown to be present in only small amounts in embryonic non-myelinated nervous tissue and which appear to be closely associated with the development of the sheath, might serve as an index for the amount of myelinated fibers in a given brain or from different parts of the same brain. This suggestion requires further study.

Material and Methods.

The material for the present study was obtained from man and dogs. The dog material, which was analyzed first, was obtained from the animals used for the laboratory classes in Physiology at the University of Chicago during the Spring Quarters of 1911 and 1912. The collection of material for each sample covered a period of about 2 weeks, as it required a large number of dogs to furnish a sufficient amount for chemical analyses, especially in the case of the nerve roots. The material at each period of collection was weighed and then put immediately into alcohol and preserved according to the methods employed in the chemical analyses of nerve tissue.[9] The ages of the animals from which the material was collected were not known. We aimed, however, to take dogs of average size, usually full grown, as this made the collecting of the nerve roots less tedious. The human material

[7] Koch, W., *Am. J. Physiol.*, 1904, xi, 306.

[8] Koch and Koch,[6] p. 443.

[9] Koch, M. L., and Voegtlin, C., *Bull. Hyg. Lab., U. S. P. H. S., 103* 1916, 67–82.

was collected in 1912 and 1914. The corpus callosum data represent duplicate analyses of one case (this was checked with other analyses made by Waldemar Koch,[10]) and the data for the intradural nerve roots represent three analyses of three individual cases.

Collection of Material.—The method employed for the collection of the corpus callosum in both man and dog was as follows: The cerebrum was divided into its two hemispheres, each hemisphere was laid with its mesial surface on a glass plate and then cut vertically into sections about 2 cm. thick. These sections were then laid on the glass plate and with a sharp scalpel the white matter was cut out, leaving a margin of white attached to the gray matter. These pieces of white matter were weighed to 0.001 gm. Care was taken to expose the material as little as possible to the air. It was therefore kept tightly covered with a glass bell jar and the collection was made as rapidly as possible.

The intradural nerve roots were collected in the following way: In the dog the spinal cord was exposed and without removing the cord from the body the lumbosacral nerve roots were cut away between their attachment to the cord and their passage through the dura. In man the entire cord with the nerve roots attached was removed from the body, with the dura intact. The dura was then opened, each nerve root picked up carefully with a pair of forceps, and cut between its two attachments. Care was taken to clip off any ganglion substance at the distal end of the nerve roots. Each nerve root was placed immediately in a glass stoppered weighing bottle and weighed to 0.001 gm. The method employed in the chemical analysis of this material is described in detail elsewhere.[9] This method in turn is based on the methods worked out and elaborated by Waldemar Koch for the analysis of brain tissue.[11]

Chemical Findings.

A comparison of the corpus callosum and the intradural nerve roots of the dog confirms the earlier observations of Hatai (Table I) as to the consistency of the ratios between the percentage of water and ether-alcohol extract in these two types of white matter (Table II). In man the percentage of water and of extract

[10] Koch, W., and Mann, S. A., *J. Physiol.*, 1907, xxxvi, 1.
[11] Koch, W., *J. Am. Chem. Soc.*, 1910, xxxi, 1329.

in the corpus callosum is in agreement with that in the dog. In the nerve roots, however, we find in man that the water is several (three) points higher and the extract is several (seven) points lower than in the dog (Table II). In this connection it may be noted that the human case, No. 17 (column C, Table V) shows the widest departure from the mean. The more detailed analysis of the individual constituents also indicates the greatest departure from the average in No. 17. The averages for the nerve roots in man were therefore based on the two remaining cases.

If in man (Table V) the determinations for the callosum are taken as the standard and those for the nerve roots compared with them, we find that the *water* and *proteins* are higher in the nerve roots, the *extractives* (organic extractives and inorganic constituents) are very much lower, while the *lipoids*, on the other hand, are in agreement.[12] For the individual lipoids we find that the *phosphatides* are slightly higher in the nerve roots, the *sulfatides* appear to be in agreement and the *cholesterol* also. The *cerebrosides* could not be compared as the small samples of the nerve roots did not permit a complete analysis to be made.

The *distribution of the sulfur in per cent of total sulfur*, in the protein, lipoid, and water-soluble fractions shows the protein sulfur to be in close agreement, the lipoid sulfur to be slightly lower, and the water-soluble sulfur to be very much higher. The *distribution of the phosphorus in per cent of total phosphorus* in the same fractions shows the protein phosphorus to be lower, the lipoid phosphorus to be slightly higher, and the water-soluble phosphorus to be very much lower. The total sulfur and the total phosphorus are practically the same in both callosum and nerve roots.

[12] In making a comparison between the values given in the accompanying tables, a definite plan has been followed. Using as a standard the value to which a second value is referred, the percentage deviation has been determined in each instance.

Values which vary from the standard used:

By less than	10	per cent	are considered in *agreement*.
From	10–20	" " " "	slightly different.
From	20–30	" " " "	different.
Above	30	" " " "	very different.

The language of comparison is not always the same, but the distinctions noted above have been carefully maintained.

TABLE V.

Man. Corpus Callosum and Intradural Nerve Roots. Proportions of the Constituents in Percentage of Solids.

	A.	B.	C.	D.	E.	F.	G.
	Corpus callosum.		Intradural nerve roots.			Corpus callosum, averages.	Intradural nerve roots, averages of No. 26 and No. 30.
Laboratory No.	W. 42 I.	W. 42 II.	17	26	30		
Case No.	Normal.	Normal.	N. I.	N. II.	N. IV.		
Weight of sample, *gm.*	47.86	39.82	8.90	6.71	6.17		
1.							
Water, *per cent.*	70.22	70.40	(72.93)	72.31	72.06	70.31	72.18
Solids, " "	29.78	29.60	(27.07)	27.69	27.94	29.69	27.82
2.							
Proteins	29.37	29.13	(38.26)	35.40	35.18	29.25	35.29
Extract	70.63	70.87	(61.74)	64.60	64.82	70.75	64.71
Extractives (organic extractives and inorganic constituents)	5.12	(5.12)	(3.21)	2.80	2.73	5.12	2.76
Lipoids	65.51	65.75	(58.53)	61.80	62.09	65.63	61.95
3.							
Phosphatides	27.78	27.48	(34.08)	35.80	33.92	27.63	34.86
Cerebrosides						(16.60)*	
Sulfatides	7.33	7.60	(5.64)	7.76	7.83	7.46	7.79
Cholesterol			(11.77)	10.64	12.41	(10.25)*	11.52
4.							
Total sulfur	0.49	0.51	(0.49)	0.53	0.57	0.50	0.55
" phosphorus	1.43	1.42	(1.55)	1.58	1.52	1.42	1.55
5.	Distribution of sulfur in percentage of total sulfur.						
Protein S	56.43	51.20	(63.60)	52.02	52.00	53.80	52.01
Lipoid S	29.91	34.90	(22.80)	29.08	27.27	32.40	28.17
Water-soluble S**	13.66	13.80	(13.60)	18.90	20.74	13.80	19.82
6.	Distribution of phosphorus in percentage of total phosphorus.						
Protein P	12.80	12.00	(10.2)	8.52	9.56	12.40	9.04
Lipoid P	75.00	76.68	(85.00)	87.56	86.70	75.80	87.12
Water-soluble P**	12.20	11.40	(4.76)	3.95	3.74	11.80	3.84

* See Table III, 3, F.

** Water-soluble S and P in this table and Tables VI, VII, and VIII represent the sulfur and phosphorus found in the extractives (2).

If in the dog (Table VI) the determinations for the callosum are taken as the standard and those for the nerve roots compared with them, then we find that the values for *water, proteins,* and *lipoids* are in agreement, those for the *extractives* (organic extractives and inorganic constituents) are, as in man, lower in the nerve roots. For the individual lipoids we find that the *phosphatides* are higher in the nerve roots and the *sulfatides* are slightly lower as in man. The cerebrosides and cholesterol were not determined and can therefore not be compared.

The *distribution of the sulfur in per cent of total sulfur,* in the protein, lipoid, and water-soluble fractions shows the protein sulfur and the water-soluble sulfur to be in close agreement, while the lipoid sulfur is slightly higher. The *distribution of the phosphorus in per cent of total phosphorus* in the same fractions shows the protein phosphorus to be lower, the lipoid phosphorus slightly higher, and the water-soluble phosphorus very much lower in the nerve roots as compared with the callosum. The total sulfur is slightly lower, while the total phosphorus is slightly higher in the nerve roots as compared with the callosum.

In order to determine from the foregoing data whether the relative amount of sheathing substance in the corpus callosum and in the intradural nerve roots in man and dog is similar we shall first have to compare the white matter from the corpus callosum of man with that of the dog and to make a similar comparison in the case of the nerve roots, and, second, we shall have to compare the white matter from the callosum (dog) with that from the nerve roots in each of these two forms.

1. When we compare (Table VII) the white matter in the callosum of man with that in the callosum of the dog, *taking the data for the dog as the standard,* we find that the agreement for the water and the individual constituents is close, except for the extractives (organic extractives and inorganic constituents), which are low, and the water-soluble sulfur, which is very much higher in man.

When we compare the white matter in the nerve roots in man with that in the nerve roots of the dog, again *taking the data for the dog as the standard,* we find the agreement less close for the water as well as for the individual constituents. In the intradural nerve roots of man, both the water and the protein are

TABLE VI.

Dog. Corpus Callosum and Intradural Nerve Roots. Proportions of the Constituents in Percentage of Solids.

	A.	B.	C.	D.	E.	F.
	Corpus callosum.		Intradural nerve roots.		Corpus callosum, averages.	Intradural nerve roots, averages.
Laboratory No.	C. 100(a).	C. 100(b).	C. 101(a).	C. 101(b).		
Year of analysis	1911	1912	1911	1912		
No. of dogs used	10	8	19	18		
Weight of sample, *gm.*	10.61	14.17	7.85	8.69		
1.						
Water, *per cent.*	70.03	70.19	70.0	68.65	70.11	69.32
Solids, " "	29.97	29.81	30.0	31.35	29.89	30.68
2.						
Proteins	31.74	29.85	31.63	25.46	30.79	28.54
Extract	68.26	70.15	68.37	74.54	69.21	71.46
Extractives (organic extractives and inorganic constituents)	6.01	6.47	4.55	4.51	6.25	4.53
Lipoids	62.25	63.68	63.82	70.03	62.96	66.93
3.*						
Phosphatides	30.35	29.60	35.48	38.94	29.97	37.21
Sulfatides	8.22	8.72	7.26	7.84	8.47	7.53
4.						
Total sulfur	0.51	0.49	0.44	0.37	0.50	0.41
" phosphorus	1.54	1.51	1.64	1.77	1.52	1.70
5.	Distribution of sulfur in percentage of total sulfur.					
Protein S	57.92	55.90	56.32	49.78	56.91	53.05
Lipoid S	32.10	35.30	32.60	42.42	33.70	37.51
Water-soluble S	9.98	8.80	11.08	7.79	9.39	9.43
6.	Distribution of phosphorus in percentage of total phosphorus.					
Protein P	11.60	12.96	10.30	8.00	12.28	9.15
Lipoid P	76.22	75.67	83.75	85.29	75.94	84.52
Water-soluble P	12.18	11.37	5.95	6.71	11.77	6.33

* Samples were too small to permit cerebrosides and cholesterol to be determined.

high, while the lipoids are slightly lower and the extractives are very much lower. We find the lipoid sulfur to be lower and the water-soluble sulfur very much higher in the nerve roots of man than of the dog, while the water-soluble phosphorus is very much lower.

2. When we compare (Table VII) with the white matter taken from the callosum that from the nerve roots, again *taking the data for the dog and the data for the callosum as standards*, we find that in the dog the agreement between these two is close, except for certain consistent differences which also appear in the nerve roots of man.

The most characteristic differences in composition, by which the nerve roots are distinguished from the callosum in both man and dog, are therefore the low extractives and water-soluble phosphorus, and the high phosphatides and lipoid phosphorus. In man, however, besides these differences, we find also a higher percentage of water and proteins and a very much higher water-soluble sulfur. From this it appears that the nerve roots of man show the greatest departure from the dog corpus callosum, which was taken as a standard throughout our comparisons, while the dog nerve roots and the human callosum are in fairly close agreement.

We find that the extractives (organic extractives and inorganic constituents), the water-soluble sulfur, and the water-soluble phosphorus show the greatest variation. These fractions, especially the water-soluble sulfur fraction, were considered by Koch[7] to bear an important relation to the protein-sulfur fraction and possibly to be of value in investigating the protein metabolism of the central nervous system during development. It seemed therefore of interest to compare the ratios of these two fractions in the corpus callosum and in the nerve roots.

A ratio between the protein-sulfur and the water-soluble sulfur (calculated in percentage of total sulfur) showed the following (Table VIII): The ratio in the callosum is the same in man as in the dog (1:4); the ratio in the nerve roots of the dog is fairly close to the callosum of both man and dog (1:5.8); while the ratio of the nerve roots of man is not at all close to that of the callosum (1:2.6), which, on the other hand, agrees quite closely to the ratio in the 2 year old callosum (1:2). These ratios therefore

TABLE VII.

Dog and Man. Corpus Callosum and Intradural Nerve Roots, Based on the Averages in Tables V and VI. Proportions of the Constituents in Percentage of Solids.

	A.	B.	C.	D.
	Corpus callosum.		Intradural nerve roots.	
	Dog.	Man.	Dog.	Man.
1.				
Water, *per cent*	70.11	70.31	69.32	72.18
Solids, " "	29.89	29.69	30.68	27.82
2.				
Proteins	30.79	29.25	28.54	35.29
Extract	69.21	70.75	71.46	64.71
Extractives (organic extractives and inorganic constituents)	6.25	5.12	4.53	2.76
Lipoids	62.96	65.63	66.93	61.95
3.				
Phosphatides	29.97	27.63	37.21	34.86
Cerebrosides*		(16.60)		
Sulfatides	8.47	7.46	7.53	7.79
Cholesterol*		(10.25)		11.52
4.				
Total sulfur	0.50	0.50	0.41	0.55
Total phosphorus	1.52	1.42	1.70	1.55
5.	Distribution of sulfur in percentage of total sulfur.			
Protein S	56.91	53.80	53.05	52.01
Lipoid S	33.70	32.40	37.51	28.17
Water-soluble S	9.39	13.80	9.43	19.82
6.	Distribution of phosphorus in percentage of total phosphorus.			
Protein P	12.28	12.40	9.15	9.04
Lipoid P	75.94	75.80	84.52	87.12
Water-soluble P	11.77	11.80	6.33	3.84

* Cerebrosides and cholesterol were not compared as these were not determined in the dog material (Table VI).

again bring out, what has already been indicated above, that the chemical composition of the nerve roots of man does not appear to be as close to the callosum as is that of the nerve roots of the dog.

TABLE VIII.

Ratio of the Water-Soluble Sulfur to Protein Sulfur in the Corpus Callosum and Intradural Nerve Roots in Man and Dog, Also in the 2 Year Old Corpus Callosum in Man.

	Dog.		Man.		Man (2 yrs.)* corpus callosum.
	Corpus callosum.	Nerve roots.	Corpus callosum.	Nerve roots.	
Protein S	56.91	53.05	53.81	52.01	63
Water-soluble S	12.28	9.15	13.80	19.82	31
Ratios	1:4	1:5.6	1:4	1:2.6	1:2

* Koch.[10]

DISCUSSION.

To interpret the foregoing results it is necessary to make a general survey of them—and for this purpose we shall use the percentage value of the extract fraction, *i.e.*, the sum of the percentage values for the lipoids plus those for the extractives (organic extractives and inorganic constituents).

For the callosum in man we have (Table V, 2, A, B) 70.63 and 70.87 per cent—the results of two analyses on the same material. From an earlier study (Table III, 2, F) the data for man at 19 years gives 72.9 per cent.

For the callosum in the dog the corresponding values (Table VI, 2, A, B) are 68.26 and 70.15 per cent—the analyses being made on different groups a year apart; Hatai had found 68.13 per cent (Table I), but, as noted, Hatai's ether-alcohol extract should not be expected to give quite so large a value as appears in our extract fraction as the latter contains only part of the extractives (water-soluble) as these were only incompletely extracted by Hatai's method.

Reserving comment on these results for the callosum we pass to the corresponding data for the intradural nerve roots.

In the case of man (Table V, 2, C, D, E) the values for the extract from the nerve roots in three different cases were 61.74,

64.60, and 64.82 per cent, the first determination being distinctly lower than the other two and not used in making the averages. In the dog, on the other hand, the values are 68.37 and 74.54 per cent (Table VI, 2, C, D) while Hatai found 69.29 per cent (Table I); see the explanation in the preceding paragraph.

Thus duplicate analyses of the same material yield in the case of the human callosum results which are very nearly alike (Table V). The differences noted above are therefore probably not due to technical errors and must arise from other causes.

It further appears that the extract fractions from the human corpus callosum differ by somewhat less than two points (1.54 per cent) from those for the dog (Table VII).

This difference probably corresponds to a difference in the amount of sheathing material in the several animals from which the samples were taken. On the average the value for the extract fraction in man (Table VII) is a trifle greater than for the dog, and in view of the fact that the lowest value found for man is above the highest value found for the dog this may mean that the sheathing substance of the callosal fibers of man is a trifle more developed, though it would perhaps be safer to conclude that in this character man and dog were nearly alike.

As regards the intradural nerve roots, the agreement is less close. While the extract fraction for the nerve roots in the dog (Table II) is 71.46 per cent or about two points (2.14 per cent) above that for the callosum, in the case of man it is only 64.71 per cent or about seven points (6.47 per cent) *below* that for the callosum.[13] In considering this difference it must be borne in mind that the human material was from subjects dead of presumably non-nervous diseases, while the dogs were killed in health. Still, were disease the cause of the difference we should be obliged to assume, either that the values as found for the callosum in man were also too low or that the pathological conditions had not modified the sheathing substance in the callosum.

Though the pathological conditions may have had some influence in bringing about this result, it seems at the moment more plausible to think of the species difference between the two animals, involving as it does a difference in the relative use of the hind limbs, as possibly the more important condition.

[13] It must be remembered that the samples for the callosum and the samples for the nerve roots were taken from different individuals.

If we should accept the sulfatides (lipoid sulfur) as an index of myelination (Table V, 5, F, G, and Table VI, 5, E, F) we should say that the two forms of white matter here compared are very close. It remains to be determined, however, whether this index holds good in other relations before we can accept it as significant.

CONCLUSIONS.

From this survey we conclude that the quantity of the sheathing substance appears to be nearly the same in the callosum fibers of man and of the dog. Moreover, the amount of sheathing substance of the fibers of the intradural nerve roots is in the dog like that found in the callosum, although a higher percentage of phosphatide and a lower percentage of water-soluble phosphorus appear to be characteristic for the nerve roots in both man and the dog. In man, however, the intradural nerve roots appear to have somewhat less sheathing substance, possibly a species difference, possibly due to some other cause.

The approximate similarity in the amount of sheathing substance formed on the peripheral and on the central nerve fibers (though the latter are devoid of neurilemma) in two of the higher mammals, and the fact that the amount of sheathing substance in the callosum of man is similar to that in the callosum of the dog—and in the case of the intradural nerve roots not so very different—are the large results to which attention is especially directed.

THE EFFECT OF DIFFERENT SALTS ON AMMONIA FORMATION IN SOIL.*

By GEORGE P. KOCH.

(From the New Jersey Agricultural College Experiment Station, New Brunswick.)

(Received for publication, June 6, 1917.)

It is generally known and has recently been proven by Tottingham and Shive with plants in nutrient solutions of controlled concentrations that the high concentrations of salts are injurious to plants, while the same combination of salts at lower concentrations did not retard their growth. Likewise the writer, controlling the concentration of the solution, studied the effects of a three salt solution (the physiological balance in nutrient solution) upon the decomposition of dialyzed peptone by a pure culture of bacteria and found the same effects. Consequently in order to rule out osmotic differences all solutions used in the work here presented were made up to an osmotic pressure of two atmospheres.

In making up the solutions from the three salts, the triangular diagram known as the Gibbs method, which was used satisfactorily by Schreiner and Skinner and by Shive, was used. An addition to this method was made, however; solutions representing combinations of salts outside the triangle were considered.

To 100 gm. quantities of a "sassafras" soil with which were mixed 155 mg. of nitrogen in the form of dried blood, different combinations of the three salts, $MgSO_4$, K_2SO_4, and $Ca(H_2PO_4)_2.2H_2O$, which were used in a previous work by the writer were applied. The mixture was incubated for a period of 8 days and the ammonia distilled off by the usual method. The determinations were made in duplicate or triplicate and in the most important solutions four determinations were made.

The total concentration of all the solutions employed was two atmospheres. Tottingham clearly discussed the method of calculating the partial osmotic concentration and molecular concentration of a series of salts in combination in order to secure a certain total concentration. The same

* This paper was prepared to be presented at the Biological Chemical Section of the American Chemical Society at its session in Kansas City, Missouri, April 10, 1917.

method was employed throughout this work. It was assumed that the degree of ionization of the salts in every combination was the same as if each were used separately.

No doubt the effect upon the total concentration of the solution when this was in the soil, due to the differential absorption of the salts, was considerable, as shown by Bouyoucos and McCool, but this matter was not brought into consideration at this time.

The following data show the effect of various combinations of a three salt solution upon the composition of dried blood in a soil when the total concentration of the salts applied is two atmospheres.

Molecular proportions of salts.			Ammonia produced.
$MgSO_4$	K_2SO_4	$Ca(H_2PO_4)_2.2H_2O$	
1	1	8	126.6
1	0	9	126.8
0	1	9	126.4
0	0	10	126.7
1	8	1	107.6
0	9	1	107.7
1	9	0	90.5
0	10	0	88.0
8	1	1	106.0
9	0	1	101.0
9	1	0	87.0
10	0	0	85.6
1	4	5	122.6
0	5	5	120.7
4	5	1	104.5
5	5	0	85.0
5	0	5	124.7
4	1	5	120.5
No treatment			100.0* (64.0)

* The amount of decomposition in the soil receiving no treatment of salts was always taken as 100.0 per cent and the decomposition in the others is expressed in terms of this. The actual amount of decomposition in mg. of N of the "no treatment" is given in parentheses.

SUMMARY.

Utilizing various combinations of $MgSO_4$, K_2SO_4, and $Ca(H_2PO_4)_2.2H_2O$, and controlling the concentration at two atmospheres, the following effects on ammonia formation from dried blood in soil were obtained.

(*a*) In combinations of the salts where $Ca(H_2PO_4)_2.2H_2O$ was present in only 0.1 of the total concentration a considerable increase in ammonia formation was apparent.

(*b*) When 0.8, 0.9, or all of the total concentration was supplied by $Ca(H_2PO_4)_2.2H_2O$ the ammonia formation was approximately 26.0 per cent greater than when no salts were added to the soil.

(*c*) $MgSO_4$ and K_2SO_4 singly or in combination were toxic where no $Ca(H_2PO_4)_2.2H_2O$ was added in the combination.

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THE BEHAVIOR OF CHICKENS RESTRICTED TO THE WHEAT OR MAIZE KERNEL.* II.

By E. B. HART, J. G. HALPIN, and H. STEENBOCK.

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Plate 3.

(Received for publication, July 9, 1917.)

The popular view prevails that among the cereal grains wheat is of superior nutritive worth. Our numerous experiments with mammals[1] point to the contrary view and leave little room for doubt that the wheat grain contains a mildly toxic material. In addition, its proteins are of inferior quality and in a measure may be responsible for some of the malnutrition we have observed where wheat was fed excessively. The fact, however, that the corn kernel proteins are equally inferior for growth, but that this grain is not otherwise deleterious to normal nutrition would most probably place the responsibility for lower nutritive value of the wheat kernel upon some inherent toxic substance or substances.

With chickens, started at half their mature normal weight, we found[2] that they could make slow growth, maintain themselves, and produce fertile eggs on rations limited to corn meal, gluten feed, and calcium carbonate, or wheat meal, wheat gluten, and calcium carbonate. These results are in marked contrast to our records with swine where either of the above rations leads eventually to loss of weight, cessation of oestrum, and in the case of wheat to a condition resembling polyneuritis.

* Published with the permission of the Director of the Wisconsin Agricultural Experiment Station.

[1] Hart, E. B., McCollum, E. V., Steenbock, H., and Humphrey, G. C., *Wisconsin Agric. Exp. Station, Research Bull. 17*, 1911; *J. Biol. Chem.*, 1912–17.

[2] Hart, E. B., Halpin, J. G., and McCollum, E. V., *J. Biol. Chem.*, 1917, xxix, 57.

The tolerance of wheat, without ill effects, and the growth and maintenance made by half grown or mature chickens when limited to this grain emphasize the difference in behavior of species in respect to resistance and nutritional requirements.

These early observations, however, left unsettled the question as to the effect of excessive wheat feeding on younger chickens and for that reason these studies were continued. In the experiments to be reported in this paper birds were chosen weighing from 2 to 3 pounds, thus giving opportunity for the animal to make considerable growth. The records secured are not in harmony with those made with nearly grown or mature chickens. The younger birds tolerate the wheat very much less effectively than do those more mature and then only when the mineral content of the ration is adjusted, the proteins of the wheat improved by the addition of casein, and a more liberal supply of the fat-soluble vitamine (from butter fat) added to the ration. Adjustment of a single nutritive factor in the case of feeding wheat to partly grown chickens was insufficient for normal nutrition, although it is evident from our records with the corn grain that the mineral requirements of the growing chicken are essentially different from those of the growing mammal. We have records (to be published later) where baby chicks have grown quite as well on the corn grain fortified only with casein and common salt as on corn grain fortified with casein, common salt, and a complex salt mixture.

On a monotonous diet the mineral content of the ration, to be physiologically balanced, must possess a rather definite qualitative and quantitative composition. Undoubtedly there may exist some latitude in this composition; but one of the most important functions of the salt mixture of a diet is to keep the epithelial cells of the digestive tract functioning normally. This is quite as important as meeting the "needs" of construction and we recognize it as one of the important problems in a study of the mineral requirements of animals on restricted and unvarying diets.

EXPERIMENTAL.

For this work vigorous pullets (Rhode Island Reds) were selected. There were three in a lot, confined to wire cages, with shavings as litter and scratch. They were placed in the cages on

October 15 and the experimental observations terminated June 25, a period of about 8 months' observation. Distilled water was used for all lots and quartz grits were placed in the hoppers. The salts were mixed intimately with the feed whether fed as dry or wet mash. Calcium carbonate was used as the precipitated carbonate in the proportion of 3 pounds to 100 pounds of air-dried feed. Other details of experimentation were precisely as described in our earlier publication.[2] The feeds used in the check lots consisted of 3 parts of corn, 2 parts of wheat, 1 part of oats as scratch, and 1 part each of bran, middlings, and corn as mash. These were supplied at the rate of 2 parts of scratch feed to 1 of mash. The records of the rations fed, growth and behavior of birds, etc., are recorded in Table I.

The striking thing to be observed in the table is the fact that at the end of 3 months' time there was a mortality of 100 per cent in the wheat-fed lots, with the single exception of the lot receiving wheat fortified with casein, butter fat, and a salt mixture. Only two birds had been lost in the three corn kernel lots. The butter fat and casein additions appeared to be the most helpful adjuvants for counteracting the depressing action of continued wheat feeding to this species. This statement rests upon the fact that the addition of a complex salt mixture to the corn kernel and its endosperm proteins did not improve it for the growth and maintenance of chickens and the mineral content of the wheat grain is not strikingly different from that of the corn kernel. The birds that died became greatly emaciated, but no other symptoms of striking character developed. However, when excited, they would often be seized with serious spasms, provoking extraordinary flapping of the wings, followed by exhaustion and collapse. This would indicate a derangement in the nervous system. Excessive wheat feeding to cattle or swine ultimately induces pathological changes in the nervous tissue.[3] It is possible that such changes were occurring here, but up to the present time no histological studies have been made.

The corn grain fortified only with its endosperm proteins and calcium carbonate suffices for growth and the continued main-

[3] Hart, E. B., Miller, W. S., and McCollum, E. V., *J. Biol. Chem.*, 1916, xxv, 239. Hart, McCollum, Steenbock, and Humphrey, *J. Agric. Research*, 1917 (in press).

TABLE I.

Record of Results 1916–17.

Duration, October 15, 1916, to June 30, 1917. Laying Period April 16 to June 23.

Ration.	Bird No.	Weight.					Date of death.	Final weight.	Eggs collected.
		Oct. 15	Dec. 15	Jan. 15	Feb. 15	June 15			
		lbs.	*lbs.*	*lbs.*	*lbs.*	*lbs.*		*lbs.*	
Corn meal 70 lbs.	801	3.06	3.43	3.43	3.62	3.5		3.62	0
Gluten feed 30 "	802	2.25	2.00	Dead.			Dec. 1916	1.50	0
$CaCO_3$ 3 "	803	2.56	3.50	3.75	4.31	3.31		3.68	8
Quartz.									
Corn meal 70 lbs.	804	2.93	3.31	3.18	3.37	2.63		2.75	10
Gluten feed 30 "	805	2.18	2.00	1.68	1.75	Dead.	Apr. 1916	1.37	0
$CaCO_3$ 3 "	806	2.16	3.18	3.16	3.37	3.43		3.43	3
K_2HPO_4 323 gm.									
Ca lactate 513 "									
Quartz.									
Corn meal 95.5 lbs.	819	3.06	4.5	4.62	5.00	4.12		4.37	
Casein 2.5 "	820	2.81	3.87	3.75	3.81	3.56		3.56	0
K_2HPO_4 323 gm.	821	2.06	2.37	2.25	2.43	2.75		3.30	6
Ca lactate 513 "									0
$CaCO_3$ 3 lbs.									
Quartz.									
Wheat meal 95.5 lbs.	807	2.68	2.5	Dead.			Dec. 1916	1.75	0
Wheat gluten 2.5 "	808	2.62	1.75	"			" 1916	1.75	0
$CaCO_3$ 3 "	809	2.25	3.31	3.00	Dead.		Jan. 1917	3.00	0
Quartz.									
Wheat meal 95.5 lbs.	810	3.25	2.87	Dead.			Jan. 1917	1.81	0
Wheat gluten 2.5 "	811	2.31	1.93	"			Dec. 1916	1.25	0
K_2HPO_4 323 gm.	812	2.31	1.75	"			" 1916	1.50	0
Ca lactate 513 "									
$CaCO_3$ 3 lbs.									
Quartz.									
Wheat meal 95.5 lbs.	813	2.75	2.25	Dead.			Dec. 1916	2.00	0
Casein 2.5 "	814	2.56	2.50	"			" 1916	2.00	0
K_2HPO_4 323 gm.	815	2.12	2.12	"			Jan. 1917	1.25	0
Ca lactate 513 "									
$CaCO_3$ 3 lbs.									
Quartz.									

TABLE I—*Concluded.*

Ration.	Bird No.	Weight. Oct. 15	Dec. 15	Jan. 15	Feb. 15	June 15	Date of death.	Final weight.	Eggs collected.
		lbs.	*lbs.*	*lbs.*	*lbs.*	*lbs.*		*lbs.*	
Wheat meal 95.5 lbs.	816	3.12	3.87	3.00	2.25	Dead.	Feb. 1917	2.25	0
Casein 2.5 "	817	2.56	4.06	4.68	5.25	4.37		4.50	6
Butter fat 2 "	818	1.87	2.50	1.75	Dead.		Jan. 1917	1.75	0
K_2HPO_4 323 gm.									
Ca lactate 513 "									
$CaCO_3$ 3 lbs.									
Quartz.									
Check, variety	822	2.75	3.37	3.00	3.18	3.5		3.00	6
grain ration.	823	2.00	Dead.				Nov. 1916	1.12	0
$CaCO_3$ 3 lbs.	824	2.50	4.00	4.25	4.37	3.43		3.50	16
Quartz.									
Milk.									
Check, variety	825	3.00	4.31	4.00	4.75	3.75		4.25	4
grain ration.	826	2.93	2.87	2.75	2.87	Dead.	Feb. 1917	2.50	0
$CaCO_3$ 3 lbs.	827	1.81	2.62	2.25	Dead.		" 1917	2.00	0
Quartz.									

tenance of this species. From these data and those published earlier[2] it is apparent that the corn kernel can supply adequately the vitamine demands (both fat-soluble and water-soluble) of growing and reproducing chickens.

Figs. 1 to 3 contrast the condition of certain of these animals after 75 days on the ration. Even after 8 months' use of these rations exactly similar conditions prevailed; the birds fed the corn grain, gluten feed, calcium carbonate ration continued to thrive, as also did one from the group of chickens fed the wheat, casein, and butter fat, and a salt mixture.

SUMMARY.

1. Chickens started below their half normal weight can make slow growth and maintain themselves on rations restricted to corn meal, gluten feed, and calcium carbonate. But a ration restricted to the wheat grain, wheat gluten, and calcium carbonate will terminate life in this species in 3 months. These re-

sults harmonize with our data relative to the mammal, particularly in respect to the action of the wheat grain.

2. These and our earlier records illustrate, however, a difference in the behavior of more mature and younger chickens. The former will tolerate wheat, but the latter, like either mature or young mammals, will not tolerate excessive wheat feeding. There is also a difference in the mineral needs of growing chickens and growing mammals.

3. Modifying the wheat grain by the addition of a complex salt mixture as a single change, or including an additional alteration, such as substituting casein for part of the wheat protein, did not appear to improve the dietary properties of this grain for young growing chickens. Only when butter fat was added and the casein-mineral modification also included was there complete tolerance and well-being.

4. These results do not mean that wheat or wheat products cannot be fed growing chickens or mammals, but only point out their dietary limitations with another species. The data also show that the wheat grain cannot be used exclusively and successfully for young growing chickens without the introduction of other dietary factors, and indicate what those factors are.

EXPLANATION OF PLATE 3.

FIG. 1. Condition of a fowl at the end of 3 months' on a ration of 70 pounds corn meal, 30 pounds gluten feed, 3 pounds $CaCO_3$, distilled water, and quartz. From all appearances this was a normal bird.

FIG. 2. After 3 months on a ration of 95.5 pounds wheat meal, 2.5 pounds wheat gluten, 3 pounds $CaCO_3$, distilled water, and quartz. Dead when photographed. Somewhat emaciated. A typical condition of exclusively wheat-fed birds without complete modification of the ration.

FIG. 3. After 3 months on a ration consisting of 95.5 pounds wheat meal, 2.5 pounds casein, 2 pounds butter fat, 3 pounds $CaCO_3$, 513 gm. calcium lactate, 323 gm. K_2HPO_4, quartz, and distilled water. Apparently normal condition, although this was the only bird in the four wheat-fed lots that survived and then only when the wheat meal, wheat gluten mixture was modified in respect to proteins, ash, and the amount of the fat-soluble vitamine.

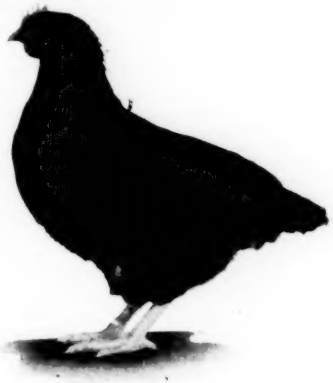

FIG. 1

FIG. 2.

FIG. 3.

(Hart, Halpin, and Steenbock: Wheat or Maize Rations.)

STUDIES IN CALCIUM AND MAGNESIUM METABOLISM.

I. THE EFFECTS OF BASE AND ACID.*

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Physiological literature contains comparatively few statistics dealing with the effect of acids and bases on the metabolism of calcium and magnesium.[1] In their well known compilation on mineral metabolism Albu and Neuberg state:

> The amount (of calcium) absorbed depends in part upon what other salts are taken with the nourishment, especially does sodium chloride increase the absorption of calcium and on the contrary the addition of alkalies diminishes it.

No figures or experiments are given in support of this statement.

The present investigation is primarily the outcome of a consideration of the claim of Dubois and Stolte that storage of calcium is dependent upon a suitable supply of alkali to the organism. By the addition of alkali carbonates to the food of children these investigators found that they could change a negative calcium balance to a positive one. They believed this outcome to be due to the neutralization, by the alkali, of the phosphoric and sulfuric acids formed in metabolism; also to a prevention of the formation in the alimentary canal of insoluble calcium soaps which cannot be utilized. According to the first consideration, by supplying alkali the unnecessary withdrawal of calcium as a

* The data in this and the two papers following are taken from the dissertation presented by Maurice H. Givens for the degree of Doctor of Philosophy, Yale University, 1917.

[1] On administration of acid see Heiss, Gaehtgens, Ruedel, Caspari, Granström, and Secchi; on administration of bases see Dubois and Stolte, Bertram, Magnus-Levy, and Gerhardt and Schlesinger.

neutralizing agent for acids formed in metabolism is averted; and in the second instance the loss of unabsorbed calcium by the bowel is prevented.

Methods.

The general plan of the present studies involves metabolism experiments in which the income and outgo and corresponding balance of various elements were ascertained under diverse conditions of diet. In preliminary experiments planned to test the available methods for the estimation of calcium it was found that because of the small amount of calcium present in dog urine, the precipitation of calcium as the oxalate, by McCrudden's method, resulted in a precipitate too fine to be filtered quantitatively. The difficulty was overcome by concentrating the urine. The acid urine was evaporated on the water bath to a small volume and then precipitated as directed by McCrudden, particular attention being paid to the reaction. The precipitate was allowed to stand over night, filtered on a blue ribbon S and S paper, washed two or three times with a 0.5 per cent solution of ammonium oxalate, dried, burned, dissolved in hydrochloric acid, filtered, and reprecipitated according to McCrudden's procedure for human urines. All filtrates were combined for magnesium estimation and treated according to the original description of the McCrudden method. In all cases the precipitate was ignited and weighed as calcium oxide. The latter was frequently dissolved in standard hydrochloric acid solution and titrated with standard sodium hydroxide solution to show that the precipitate was uncontaminated.

The calcium and magnesium in the ash of foods and feces were determined by McCrudden's procedure.

Phosphorus was estimated in urine by titration with uranium solution, and in the ash of feces and dried milk by the gravimetric molybdic method.

Nitrogen was determined in all cases by a Kjeldahl method.

The meats fed were bought in large quantities, cleaned free from visible fat and fascia, hashed, weighed out in 250 gm. packages, wrapped in paraffined paper, frozen, and kept in storage at 30°F. or lower. All meats kept perfectly under these conditions.

For the first set of experiments thoroughly dried powdered bread was used. Later cracker meal was substituted.

The dried skimmed milk was Merrell-Soule Company's "Klim."

Dogs B and F were catheterized every 48 hours with due antiseptic precautions. There was never a sign of cystitis. From the other dogs the spontaneously voided urine was collected at definite intervals.

Feces were marked off by feeding approximately 0.5 gm. of ground cork.

TABLE I.

Analyses of the Components of the Diets.

	Water.	N	Fat.	Ash.	CaO	MgO	P
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Meat H_1	58	2.57	19.5	0.63	0.035	0.021	
" 2	72	3.55	(2.8)	1.03	0.011	0.033	
" 3	74	3.54	(2.8)	1.04	0.009	0.037	
" 4	74	3.84	(2.8)	1.10	0.008	0.036	
" H_2	63	2.77	15	0.75	0.032	0.020	
" 5	73	3.38	(2.8)	1.03	0.011	0.037	
Dried bread		2.15	0.5	1.95	0.092	0.047	
Cracker meal 1	(9.0)	1.76	(6.0)	2.43	0.038	0.031	
" " 2	(9.0)	1.66	(6.0)	2.30	0.036	0.034	
Dried skimmed milk (Klim)		5.66		8.01	1.703	0.384	0.85
Agar-agar				11.93	1.03	0.41	

New Haven tap water of October 9, 1916, contained SiO_2 0.006, CaO 0.024, and MgO 0.004 gm. per liter.

The values in parentheses are calculated from the tables of Atwater and Bryant.

Composition of Daily Diets.

Diet.	N	Fat.	Carbohydrate.	CaO	MgO	P*	Estimated calories.**
	gm.	*gm.*	*gm.*	*mg.*	*mg.*	*mg.*	
A	8.57	48	78	186	100	617	994
B	10.10	51	51	223	170	602	952
B1	10.10	47	60	405	207	710	952
B2	10.08	51	51	160	155		952
B3	10.01	51	51	158	158		952
B4	9.61	51	51	162	158		952
B5	10.43	50	36	148	149		872
A2	7.50	38	25	200	103		648

* All phosphorus figures are taken from Sherman and Gettler except those of the dried skimmed milk.

** Calories estimated from the tables of Atwater and Bryant.

EXPERIMENTAL.

If alkali or acid influences either the nitrogen, calcium, magnesium, or phosphorus metabolism it should be revealed by adding these substances to calcium-poor and calcium-rich diets. Accordingly such experiments were carried out on two bitches.

Dog B weighed 13.2 kg. at the beginning of the experiment (October 16, 1916) and 14 at the end (March 2, 1917). Dog F weighed 14.6 kg. at the beginning of the experiment (November 21, 1916) and 16.6 at the end (March 2, 1917). Table I gives the composition of the food intakes. Agar was added to the food mixture to produce a daily evacuation of the bowel in order to lessen the possibility of reabsorption of lime. It also served the purpose of producing well formed stools. There was no diarrhea.

Unfortunately the addition of agar had been going on for some time before it was realized that this substance contains considerable calcium (about 1 per cent). The water intake was limited. The amount of sodium bicarbonate given was approximately 0.5 gm. per kilo. The exact amount fed is indicated in Tables II and III, likewise the exact amount of hydrochloric acid, introduced into the stomach by sound. The average daily results are included in Tables II and III.

Balance of Nitrogen.

In the case of Dog B there was a positive *nitrogen* balance for all periods except the last three. The final negative nitrogen balance may be due to the fact that in Period 20 the dog received 5.1 gm. of calcium lactate daily for 6 days. This may have upset the animal as the usual dose of calcium lactate for man is 0.5 gm.,[2] while a dose of 20 gm. daily will produce untoward symptoms.[3] It may be that the dog's negative nitrogen balance was due to the fact that she had been kept in the cage for such a long time.

The experiments on Dog B with dried skimmed milk, alkali, and calcium lactate were repeated on Dog F (Table III). The

[2] Sollman, T., A Manual of Pharmacology, Philadelphia, 1917.
[3] Towles, C., *Am. J. Med. Sc.*, 1910, cxl, 100.

TABLE II.

Nitrogen, Calcium, Magnesium, and Phosphorus Metabolism, Averages per Day. Dog B.

Period.	Intake.		Urine.				Feces.				Balance.			
	Standard diet.	Daily additions.	N	Ca	Mg	P	N	Ca	Mg	P	N	Ca	Mg	P
			gm.	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*gm.*	*mg.*	*mg.*	*mg.*
1	A		6.87	15	28	370	680	315	45	86	+1.01	−196	−14	+160
2	A	6.5 gm. $NaHCO_3$.	6.55	11	28	356	357	115	41	43	+1.66	+9	−8	+220
3	B		7.76	9	33	458	256	85	37	35	+2.09	+26	+31	+110
4	B	6.5 gm. $NaHCO_3$.	8.38	11	34	480	607	179	70	74	+1.11	−73	−18	+50
5	B		8.26	9	31	464	570	186	59	76	+1.27	−80	−3	+60
6	B_1	0.34 gm. CaO in milk.	8.36	9	25	450	455	288	65	116	+1.25	+64	+34	+110
7	B		8.39	11	39	476	633	172	69	66	+1.08	−64	−21	+60
8	B_1	0.34 gm. CaO in milk + 6.5 gm. $NaHCO_3$.	9.06	9	35	320	491	322	65	143	+0.55	+36	+24	+250
9	B		8.99	9	36	336	542	150	75	46	+0.56	−36	−23	+220
10	B	About 1.5 gm. HCl.	8.56	25	45	460	432	136	70	51	+1.10	−50	−28	+90
11	B		8.39	9	31	216	592	178	90	80	+1.12	−71	−4	+300
12	B_1	0.34 gm. CaO in milk + 1.5 gm. HCl.	8.88	19	28	240	485	293	109	160	+0.73	+43	−12	+310
13	B		9.39	9	29	215	—	—	—					
14	B_2	6.5 gm. $NaHCO_3$	8.91	10	27		487	150	49		+0.68	−43	+17	
15	B_2	6.5 gm. $NaHCO_3$ 0.1–0.3 gm. CaO as Ca lactate.	9.04	17	38		547	288	69		+0.50	−43	−15	
16	B_{2+3}	6.5 gm. $NaHCO_3$ 0.4–0.6 gm. CaO as Ca lactate.	9.08	19	31		547	485	54		+0.44	−36	+9	
17	B_3	6.5 gm. $NaHCO_3$ 0.7 gm. CaO as Ca lactate.	8.84	17	24		495	558	74		+0.67	+40	−6	
18	B_3	"	8.96	22	32		350	565			+0.70	+21		
19	B_{3+4}	0.7 gm. CaO as Ca lactate.	9.30	14	34		461	615			+0.08	−21		
20	B_4	1.0 gm. CaO as Ca lactate.	10.16	15	37		481	630			−1.03	+185		
21	B_4	6.5 gm. $NaHCO_3$ 1.0 gm. CaO as Ca lactate.	9.81	22	24		565	728			−0.77	+71		
22	B_4		10.07	12	36		400	140			−0.86	−43		

TABLE III.

Nitrogen, Calcium, Magnesium, and Phosphorus Metabolism, Averages per Day. Dog F.

Period.	Intake.		Urine.				Feces.				Balance.			
	Standard diet.	Daily additions.	N	Ca	Mg	P	N	Ca	Mg	P	N	Ca	Mg	P
			gm.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	gm.	mg.	mg.	mg.
1	B		8.26	35	25	326	450	200	68	70	+1.39	−117	−6	+225
2	B	7.25 gm. $NaHCO_3$.	8.43	33	26	219	740	300	97	74	+0.43	−217	−36	+330
3	B		8.81	33	28	182	680	272	80	103	+0.61	−190	−21	+330
4	B_1	0.34 gm. CaO in milk.	9.62	31	28	185	800	573	125	223	−0.32	−243	−28	+330
5	B		9.51	37	30	210	630	272	81	109	−0.04	−196	−24	+300
6	B_1	7.25 gm. $NaHCO_3$ 0.34 gm. CaO in milk.	9.43	31	29	192	670	450	87	188	−0.002	−123	+8	+450
8	B_2		8.54	30	19		660	258	45		+0.88	−173	+29	
9	B_2	0.1–0.3 gm. CaO as Ca lactate.	8.32	31	21		760	415	82		+1.00	−186	−10	
10	B_2	0.4–0.6 gm. CaO as Ca lactate.	8.36	32	19		690	586	80		+1.00	−143	−4	
11	B_3	0.7 gm. CaO as Ca lactate.	8.36	33	15		630	658	71		+0.97	−79	+9	
12	B_3	"	8.52	34	16		710	708			+0.77	−129		
13	B_{3+4}	0.7 gm. CaO as Ca lactate. 6.5 gm. $NaHCO_3$.	8.63	32	17		890	790			+0.36	−214		
14	B_4	1.0 gm. CaO as Ca lactate. 6.5 gm. $NaHCO_3$.	8.66	33	18		740	885			+0.21	−93		
15	B_4	1.0 gm. CaO as Ca lactate.	8.59	32	17		790	850			+0.26	−57		
16	B_4		8.84	27	22		640	220			+0.13	−7		

basal meat-cracker meal diet was essentially the same as that of the preceding series. The *nitrogen* balance was favorable throughout except in Periods 4, 5, and 6 when the animal, unaccustomed to the cage, developed a slight disability which was promptly relieved by a few days transfer to a large pen. As evidence of good health the continued gains in body weight over a period of 101 days may be cited.

Balance of Calcium.

On the basal meat-cracker meal or calcium-poor diet Dog B was constantly in negative calcium balance with one exception. This was near the beginning of the experiment (Period 3) and probably before the dog was thoroughly drained of its reserve (?) of lime.

After the addition of sodium bicarbonate to the basal diet A one would have expected an approach to a positive calcium balance, if the argument of Dubois and Stolte were tenable. In only one instance (Table II, Period 2) did this occur; and then we believe it was due to the meat used. (In Periods 1 and 2 there was used with Dog B a Hamburg hash purchased in the open market. Such a meat is liable to have bone ground in with it. This conclusion is confirmed if one refers to the calcium content of hashes H_1 and H_2 in Table I and then compares them with the calcium content of the other meats which were more carefully prepared under our direction. No bones were sawed in these latter meats and all tendons and fat were carefully removed.) We cannot consider this slight positive balance of 55 mg. of calcium oxide in 6 days of especial significance, further than to say that the dog was in calcium equilibrium. The methods of marking off the feces are still inevitably too crude to permit of nice discriminations involving a few mg. of calcium out of a total of several hundred eliminated per day.

When the experiment with sodium bicarbonate was repeated (Table II, Period 4) the dog showed a distinct negative calcium balance daily amounting to almost half of the fecal excretion of lime.

Inasmuch as the alkali had no effect on the calcium utilization of the meat-cracker meal diet the effect of hydrochloric acid in-

troduced by sound into the stomach was next tried. The dog was fed as usual and in 1 to 1½ hours approximately 1.5 gm. of hydrochloric acid were introduced. The dog retained the acid without any apparent untoward signs. The acid produced no effect on the balance of calcium which was negative in about the same magnitude as in the preceding and following periods. The acid did, however, have the striking effect of increasing the urinary calcium about 200 per cent (Table II, Period 10) presumably diverting some of the lime from the intestinal path.

In view of the essentially negative influence of base and acid on calcium metabolism, during a calcium-poor diet, it seemed desirable to repeat the experiment during an increased dietary calcium intake. Accordingly dried skimmed milk was substituted in nitrogen equivalent for the cracker meal, and the caloric intake was maintained constant by the addition of sucrose to the food. In this way the lime intake of the preceding diet was more than doubled.

When 0.34 gm. of calcium oxide, contained in 21.7 gm. of dried skimmed milk, was added to the diet, the negative calcium balance of the meat-cracker meal régime was turned into a distinctly positive one. When further 6.5 gm. of sodium bicarbonate were daily added to the milk ration, the positive calcium balance decreased one-half. Exactly the same results as on the milk plus alkali were obtained by introducing into the dog's stomach 1.5 gm. of hydrochloric acid. To recapitulate: the milk changed a negative to a positive lime balance; this balance was not increased by either acid or alkali, but conversely, reduced about half by both.

The addition of the lime in the milk period produced no effect on the calcium content of the urine, but of course increased the fecal output. As on the basal diet, the addition of the acid to the food nearly doubled the urinary calcium. Relatively, the increase in the lime of the urine was large, but absolutely it was small (15 to 23 mg. per day).

Following this lead the basal calcium-poor diet was next supplemented with a soluble calcium salt—calcium lactate (Merck's soluble). The initial dose of 510 mg. of air-dry calcium lactate on analysis yielded 100 mg. of calcium oxide. This was increased every other day by 510 mg. until the dog was receiving 3.57 gm.

of calcium lactate per day for 18 days and then 5.10 gm. daily for 12 days. Except in Periods 14, 20, and 22 the dog also received daily 6.5 gm. of sodium bicarbonate. Carbonates were always present in the urine during the periods of alkali administration.

An inspection of the results recorded in Table II shows that between the limits of 0.51 and 3.06 gm. of calcium lactate intake per day, with the addition of 6.5 gm. of sodium bicarbonate daily there remained a negative calcium balance of about the same magnitude as in Period 14 on the basal diet alone. Only when the daily calcium intake was raised to 3.53 gm. of calcium lactate plus the sodium bicarbonate was there a slight positive calcium balance.

It might appear from the results in Period 19, where a slight negative calcium balance occurred in the absence of alkali administration despite a daily intake of 3.53 gm. of calcium lactate, that the alkali actually facilitated the storage of lime. This interpretation is unlikely, however, since on the higher intake of calcium in Periods 20 and 21 no advantage whatever appeared in respect to calcium balance when alkali was administered. In fact the calcium balance on lime alone (Period 20) was more favorable than when alkali was furnished (Period 21).

A survey of Table II supports the conclusion that the addition of sodium bicarbonate has not had any marked influence upon the calcium metabolism. The striking fact is that 0.34 gm. of calcium oxide per day in 21.7 gm. of dried skimmed milk, regardless of base or acid, will produce a positive calcium balance; whereas 1 gm. of calcium oxide in the form of calcium lactate is necessary to accomplish the same end. The problem of the relative advantage of the combination in which calcium is fed is thus raised anew.

In the case of Dog F it is interesting to note that, for a period of 52 days, the dog had a positive nitrogen balance of 31.6 gm. and a negative calcium oxide balance of 8.7 gm., even though she was receiving extra lime on all except 10 days. In this animal the addition of dried milk or calcium lactate, each alone or with alkali, failed to convert the negative calcium balance of the basal diet into a positive one. *Here again the alleged favorable influence of alkali upon calcium storage was missed.*

Balance of Magnesium.

The magnesium balance of Dog B on the first basal diet A was negative and was not changed by the addition of alkali. When the basal diet B was instituted there was a change to a temporary positive magnesium balance due presumably to the increased intake of magnesium in the meat. Subsequently it became negative until milk was introduced into the food. The alkali did not essentially alter the magnesium balance with any of the diets used.

When hydrochloric acid was superimposed upon the dried skimmed milk diet the magnesium balance became negative. The positive daily magnesium balance upon the skimmed milk either without or with alkali is greater than the daily increment of magnesium in the milk. This suggests determinative factors other than the magnesium *per se*. The same features appear in the record of Dog F.

The variability of the magnesium excretion, when calcium salts are added to the diets, was such that one cannot draw a conclusion as to whether the increased intake of lime altered the excretion of magnesium or not.

Balance of Phosphorus.

The phosphorus balance was always positive though extremely variable quantitatively. The figures for both the urinary and fecal phosphorus were so variable that neither could be used as an index of any influence exerted by sodium bicarbonate administration. The increased phosphorus intake derived from the milk was eliminated in part by the kidneys and in part by the intestines.

One would expect on the basis of available information[4] that more phosphorus would be eliminated through the intestines when alkali is included in the diet and through the kidneys when acid is administered. In general this has proved to be the case in the present experiments.

[4] For a review of the literature of phosphorus compounds in animal metabolism see the compilation of Forbes, E. B., and Keith, M. H., *Ohio Agric. Exp. Station Technical Bull. 5*, 1914.

The Relation of Calcium to Magnesium.

The relation of calcium to magnesium has been studied by several investigators. The antagonistic action of calcium to magnesium anesthesia has been clearly shown by Meltzer and his collaborators.

Malcolm has presented considerable evidence to show that the ingestion of soluble magnesium salts causes a loss of calcium in adult dogs and hinders its deposition in young growing rats, while soluble calcium salts do not in the same way promote the excretion of magnesium.

Mendel and Benedict observed that the injection of calcium salts was followed by an increased elimination of magnesium in the urine; similarly the injection of a magnesium salt increased the output of calcium.

Tereg and Arnold found, after feeding acid calcium phosphate to dogs, a slightly increased amount of urinary calcium.

Ruedel observed an increasing urinary output of calcium in rabbits and dogs after the injection subcutaneously of calcium acetate.

Hart and Steenbock found that the addition of magnesium salts to the feed of a pig increased its urinary calcium output. The fecal calcium excretion was not influenced.

In our experiments the relation of calcium to magnesium in the urine in the case of Dog B was quite variable; for example, on the basal diet the ratio was 1:2, 1:3, and 1:4. When alkali was added to the basal diet, the Ca:Mg relation was still 1:2.5 and 1:3. The addition of dried skimmed milk did not alter this. The highest output of magnesium was during the dried skimmed milk plus alkali régime, Ca : Mg = 1:4. When acid was superimposed upon the basal diet and the basal plus dried skimmed milk the relation of calcium to magnesium was about 1:1.5 in both cases. This was due to an increased excretion of lime induced by the acid. The magnesium values were too variable to permit of drawing conclusive inferences.

The relations for Dog F were more consistent. The Ca:Mg ratio, in the majority of cases was about 1:1. This relation was not disturbed by the addition of dried skimmed milk to the diet or by the addition of alkali to either the basal diet or the basal diet plus milk. When calcium lactate or alkali and calcium lactate were added to the diet the ratio was changed to 1.5 : 1; that is to say, under these conditions more calcium than magnesium was excreted through the kidneys.

The Urinary Excretion of Calcium and Magnesium in Human Diabetes during Sodium Bicarbonate Ingestion.

An opportunity was offered to study the urinary calcium and magnesium output of a male diabetic[5] who received 40 gm. of sodium bicarbonate per day. The analytical data and the daily diet are included in Table IV. The urine of the subject always contained an excess of carbonates. If the alkali had had a significantly depressing effect upon the urinary output of calcium, the amount excreted would certainly have been less than here reported.

TABLE IV.

Analyses of the Daily Urinary Excretion of a Diabetic Man. Initial Weight 125 Pounds.

Date.	Volume.	Reaction to litmus.	Total N.	CaO	MgO	P
Nov.	*cc.*		*gm.*	*gm.*	*gm.*	*gm.*
16	3,300	Alkaline.	18.8	0.76	0.16	1.34
17	3,000	"	20.0	0.72	0.18	1.48
19	2,950	"	19.3	0.74	0.14	1.48
20	3,500	"	22.8	0.72	0.16	1.56
21	3,400	"	23.6	0.75	0.18	1.44
Average	3,230		20.9	0.74	0.16	1.46

During the above period the patient received daily 40 gm. of $NaHCO_3$ and the average daily food consumption was as follows.

	gm.		*cc.*
Casoid flour	55.5	Olive oil	8
Bran (washed)	15–30	Vinegar	3
Eggs	95	Beef extract	2–3
Cooked meat, beef, ham, fish	180–200		*gm.*
Lettuce	30	Oleomargarine (in casoid biscuit)	18
Celery (thrice boiled)	15–30	Lard (in casoid biscuit)	12
Cauliflower (thrice boiled)	150–180		
Butter	75–90		

Nelson and Burns found the normal urinary calcium oxide excretion in man to range between 0.18 and 0.62 gm. per day. *In our diabetic the alkali seems not to have influenced the urinary calcium output.*

[5] This case is included in the paper of Underhill, F. P., *J. Am. Med. Assn.*, 1917, lxviii, 497.

That the balance of calcium in the diabetic ordinarily is negative seems to be established from the recent work of Kahn and Kahn who made careful analyses of the intake and output of five diabetics. The literature of the subject is given in their paper.

SUMMARY.

Administration of base or acid produced no significant effect upon the balance of nitrogen, calcium, magnesium, and phosphorus in the dog.

Administration of hydrochloric acid increased the urinary excretion of calcium and thereby altered the relation of calcium to magnesium in the urine.

The calcium contained in milk was more effective than soluble calcium lactate in producing calcium retention.

Administration of large doses of alkali bicarbonate to a human diabetic did not decrease the urinary output of calcium.

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STUDIES IN CALCIUM AND MAGNESIUM METABOLISM.

II. THE EFFECT OF DIETS POOR IN CALCIUM.

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Albu and Neuberg have stated that the kind and intensity of calcium exchange is extraordinarily dependent upon the quality of the nourishment.

Forster fed a dog washed meat and showed by analysis of blood and tissues that the dog had lost calcium. The loss from the tissues did not equal the excess of output over intake, therefore the bones must have yielded some calcium.

Perl found a normal urinary calcium excretion in the dog on a calcium-poor diet of bread and a little condensed milk. The output was increased on a heavy meat-lard diet.

Goitein found that rabbits on a calcium-rich diet of oats and bone meal put on calcium and magnesium; with a diet less rich in calcium, as oats alone, the animals were in calcium and magnesium balance; and with a calcium-poor diet, as maize alone, the rabbits might be in nitrogen and magnesium balance but negative calcium balance.

Boekelman and Staal studied the effect of a calcium-poor diet and a calcium-rich diet on calcium excretion in man. The calcium-poor diet was an ordinary mixed ration while the comparison diet had its calcium content a little more than trebled by the addition of a liter of milk. In their four subjects the urinary calcium increased on the calcium-poor diet and decreased on the calcium-rich diet.

Patterson fed rabbits on oat-meal and maize, a diet which, as he says, "admittedly leads to calcium starvation." He found the amount of calcium in the blood in a normal proportion to the rest of the ash; in the bones, however, the relation of the calcium to the total minerals was reduced. He believes: "The bones can, without doubt, act as reservoirs of calcium (and probably of magnesium)."

The most intensive study made of the effect of diet on the calcium and magnesium excretion of dogs is that of Kochmann and his pupil Petzsch. From a study of the effects of protein, fat, and carbohydrate on the alkali-earth metabolism of dogs they concluded that the calcium balance was

apparently influenced by the amount of these organic nutrients, in that an addition of any one of the three substances to the basal ration would draw out lime from the organism in a noticeable degree. They thought the amount of calcium necessary for a maintenance of calcium balance depended upon the character of the food intake and had to be determined for each diet. Unlike the case of calcium metabolism they found magnesium exchange unaffected by protein, fat, and carbohydrate. The phosphorus metabolism likewise is believed to be influenced by the protein intake. These workers did not use a mixed diet. Protein, fat, and carbohydrate respectively were superimposed upon a diet of protein alone. In our experiments we have endeavored to provide a constant caloric intake on a mixed diet.

TABLE V.*

Urinary Alkali-Earth Excretion, Mg. per Kg.

Weight of dog.	CaO	MgO	Diet.	Author.
kg.				
11	0.7	0.8	No food.	Mendel and Benedict.
11	1.5	3.0	" "	Givens.
14	0.5	1.2	Ca-poor.	Mendel and Benedict.
20	1.0	2.5	"	Gaehtgens.
14	1.0	4.0	"	Givens.
20	1.5	1.8	"	Gaehtgens.
15.5	1.8		"	Tereg and Arnold.
16.4	2.0	4.0	"	Kochmann.
10.3	2.2	8.8	"	"
13.5	2.5		"	Ruedel.
24	2.6	5.0	"	Secchi.
13	3.0	3.2	"	Givens.
14	3.0	7.5	"	"
9.3	3.0	7.6	"	Kochmann.
4	3.0	10	"	Heiss.
14.5	3.2	3.2	"	Givens.
12	3.5	4.1	"	"
5.3	5	10	"	Kochmann.
33	1.3		Ca-rich.	Tereg and Arnold.
22	1.5		"	Perl.
7.7	3.4	8	"	Malcolm.
9.6	4	5	"	"
6.5	4.6	12	"	Kochmann.
14	6	7	"	Secchi.
7	16	13	"	Kochmann.

* The tables are numbered consecutively through the three papers.

The results of Kochmann are included in the following résumé (Table V) of all of the available literature on the urinary alkali-earth excretion of *adult* dogs. The statistics fail to show any *definite* relation between the diets and urinary calcium and magnesium. The tendency is for the excretion of these elements to increase as the intake of them increases.

From the literature on the subject we are led to conclude that a diet poor in calcium is not conducive to a storage of either calcium or magnesium despite an abundance of nitrogenous food. This is further established by our own investigations.

The fact that the body may show a satisfactory nutritive balance with respect to one essential element while being depleted of another deserves more emphasis than it has received in the past.[1] There are available for comparison in our series of experiments, the results obtained on five animals; *viz.*, Dogs B,[2] F,[3] J,[4] FL,[5] and M_2 (Table VI). With Dogs B, F, and M_2 there re-

TABLE VI.

Nitrogen, Calcium, and Magnesium Metabolism, Average Daily Analyses. Dog M_2.

Period.	Diet.	Urine.			Feces.			Balance.			Weight.
		N	Ca	Mg	N	Ca	Mg	N	Ca	Mg	
		gm.	*mg.*	*mg.*	*gm.*	*mg.*	*mg.*	*gm.*	*mg.*	*mg.*	*kg.*
1	A_2	5.98	34	49	0.60	270	30	+0.91	−159	−17	13.8
2	"	5.41	23	45	0.72	220	26	+1.30	−99	−11	13.8
3	"	5.52									14.0
4	"	5.46	36	55							14.2
5	"	5.07	33	47							14.3

sulted from the calcium-poor diets a negative balance of calcium and magnesium. The relation of calcium to magnesium in the urine was not altered appreciably in any of the dogs. Whether this would have been the case if the experiments had been con-

[1] Illustration of the significance of this will be found in the compilation of Forbes and others, *Ohio Agric. Exp. Station Bull. 295*, 1916, and *Bull. 308*, 1917.

[2] Table II, p. 425.

[3] Table III, p. 426.

[4] Table VII, p. 442.

[5] Table VIII, p. 442.

tinued over a sufficiently long period cannot be foretold. The excretion of lime in the feces was reduced owing to the decreased intake of the element.

No apparent relation between nitrogen and calcium output is evident in any of the dogs. In almost every instance the nitrogen balance has been positive while the calcium balance has been negative. This might be expected from the nature of the diet, which, though rich in nitrogen was poor in calcium.

The relation between the outputs of calcium and magnesium on the diets here under discussion is by no means constant. Thus with Dog B the urinary magnesium excretion was greater than the calcium. This decided difference may be due to the fact that this old laboratory dog had probably been on a calcium-poor diet for years, receiving no more lime than was actually needed to maintain her health.

The relation of calcium to magnesium in the urine was almost always about 1:1 in all of the other animals. Calcium always exceeded magnesium in the feces as 2:1 or more in both Dogs B and F. These two animals excreted the most of their phosphorus through the kidneys and its variations did not seem to influence the calcium output.

From the data submitted it is evident that *diets poor in calcium are not conducive to positive calcium* balance even when an abundance of nitrogenous food is available.

My thanks are due Professor Lafayette B. Mendel for his advice and criticism.

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STUDIES IN CALCIUM AND MAGNESIUM METABOLISM.

III. THE EFFECT OF FAT AND FATTY ACID DERIVATIVES.

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(Received for publication, June 20, 1917.)

The influence of fat on the mineral metabolism is of prime importance in the nutrition of growth. Steinitz has shown that in infants a high fat diet may lead to a negative balance of the alkalis and a reduction of the alkali earths. On a diet rich in cream the absorption of calcium dropped from 76 to 34 per cent.

Cronheim and Mueller have shown that in children on an ordinary milk diet the fecal calcium is not solely combined as soaps. Some of it is excreted in this form but there is a great excess in other compounds. In other words, on a moderately rich fat diet only a small amount of the calcium is removed by the fatty acid.

Bahrdt has confirmed the statement of Cronheim and Mueller.

Rothberg found that a nourishment rich in fat gave a negative calcium balance. In the same children Birk found that the magnesium balance was also negative.

Meyer says that the results of Steinitz and Rothberg and Birk are not contradictory, but that the condition of the child greatly influences the results; as, for instance, if there were a diarrhea there be would less chance for absorption in the intestines.

In experiments referred to in Paper II of this series Kochmann found that addition of lard or dextrose to a diet of protein alone increased the urinary calcium excretion. From his study of the influence of protein, fat, and carbohydrate he concluded that calcium balance was dependent upon the kind and amount of nourishment.

Experiments which were being conducted in the laboratory by Dr. J. F. Lyman on the utilization of certain fats, fatty acids, and their derivatives offered a further opportunity to study the calcium excretion under these conditions.

Two male dogs were used, so that all experiments were made in duplicate. In the control periods the dogs received a standard diet of 250 gm. of clean beef, 50 gm. of cracker meal, 40 gm.

TABLE VII.*

Nitrogen and Calcium Metabolism, Averages per Day.—Dog J.

Period	Substituted for lard.	Urine.			Feces.		Balance.		Fat utilization.**
		N	Ca	Mg	N	Ca	N	Ca	
		gm.	mg.	mg.	gm.	mg.	gm.	mg.	per cent
1	——	8.83	19	18	0.60	257	+1.00	−171	95
2	Ethyl palmitate.	8.68	20	18	1.33	393	+0.41	−314	57
3	——	9.61	32	24	0.69	329	+0.07	−257	97
4	Glyceryl palmitate.	7.99	23	25	0.51	364	+1.93	−214	93
5		9.97	34	30	0.65	329	−0.19	−264	93
6	Palmitic acid.	9.15	27		0.76	472	+0.52	−400	81
7		8.89	31		0.66	72	+0.88	−485	97
8	Glyceryl palmitate + Ca lactate.	11.74	27		0.52	122	−1.82	+107	92
9		9.08	21		0.62	307	+0.73	−228	98
10	Starch and sucrose.	10.23	16		0.61	221	−0.44	−168	

* The tables are numbered consecutively through the three papers.

** My thanks are due Dr. J. F. Lyman for the use of these unpublished figures.

TABLE VIII.

Nitrogen and Calcium Metabolism, Averages per Day.—Dog FL.

Period.	Substituted for lard.	Urine.			Feces.		Balance.		Fat utilization.*
		N	Ca	Mg	N	Ca	N	Ca	
		gm.	mg.	mg.	gm.	mg.	gm.	mg.	per cent
1		10.12	11	20	0.70	300	−0.40	−214	94
2	Ethyl palmitate.	9.08	17	22	0.91	285	+0.44	−207	48
3		9.47	29	25	0.84	336	+0.15	−264	96
4	Glyceryl palmitate.	8.68	29	35	0.62	350	+1.13	−278	93
5		9.14	33	33	0.34	378	+0.64	−314	93
6	Palmitic acid.	9.25	29		0.67	492	+0.50	−428	79
7		9.84	37		0.76	705	+0.17	−700	95
8	Glyceryl palmitate + Ca lactate.	9.11	26		0.64	1,380	+0.68	−50	88
9		9.11	31		0.53	307	+0.79	−236	98
10	Starch and sucrose.	8.52	26		0.77	336	+1.13	−257	

* My thanks are due Dr. J. F. Lyman for the use of these unpublished figures.

of lard, 10 gm. of agar, and 400 cc. of tap water. In the other periods an equal quantity of ethyl palmitate, glyceryl palmitate, palmitic acid, and glyceryl palmitate plus calcium lactate (8 gm. daily for 2 days, then 10 gm. daily for 2 days) respectively were substituted for the lard. In the last period the calorie equivalent of 40 gm. of fat was replaced by a mixture of cooked starch and sucrose. The results are summarized in Tables VII and VIII.

The present conception of the digestion and utilization of fats and other comparable esters of fatty acid would lead one to expect that if they are hydrolyzed in the normally functioning alimentary tract the resulting fatty acid will either be absorbed promptly or excreted as insoluble soap with the feces. The extent to which absorption occurs may therefore depend not only upon the digestion of the esters but also upon the degree to which alkali earths are simultaneously present in the intestine to render the fatty acids insoluble and unutilizable. Conversely the loss of alkali earths through the bowel may likewise be promoted by the presence of large quantities of fatty acids. The extent of digestion and utilization of palmitic acid and its derivatives in Dr. Lyman's experiments will be recorded elsewhere. A study of the data here presented shows, with respect to the deportment of the calcium, that when the utilization is poor the loss of calcium is proportionately larger. This is exemplified as a rule in the following data selected from the tables.

TABLE IX.

Relation of Calcium Excretion to Fat Utilization.

	Fat in food.	Fat utilization.	Daily Ca output in feces.
		per cent	*mg.*
Dog J.	Lard.	95	257
	Ethyl palmitate.	57	393
	Lard.	93	329
	Palmitic acid.	81	472
Dog F.	Lard.	94	300
	Ethyl palmitate.	48	285
	Lard.	93	378
	Palmitic acid.	79	492

Similarly the negative calcium balance was smaller in those cases where the utilization of the fat was more satisfactory.

Although the quantity of calcium fed as calcium lactate in one of the periods was undoubtedly sufficient to induce a storage of lime on the basal diet (Table VII, Period 8) this could not be accomplished when the fat utilization was poor (Table VIII, Period 8).

It is evident from the data presented that poor utilization of fats or fatty acids may increase the excretion of lime in the feces and prevent the storage of calcium even when the calcium intake is comparatively abundant.

My thanks are due Professor Lafayette B. Mendel for his advice and criticism.

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THE RELATION OF THE QUALITY OF PROTEINS TO MILK PRODUCTION. III.*

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(Received for publication, July 2, 1917.)

In 1915[1] we presented data showing marked inequality in the efficiency of the protein mixture of rations for milk production. This difference in efficiency we attached to the character and quantitative proportion of the proteins constituting the mixture. We emphasized the fact that the mammary gland, in its constructive capacity for milk proteins, was not independent of the quality of the proteins in the ration and further that the "nutritive ratio" or plane of protein intake may be varied with variation in the source of the proteins. With milk proteins constituting about 70 per cent of the proteins of the ration for a cow—the remaining nitrogen coming from the roughage, corn stover—it was possible to maintain a positive nitrogen balance and the production of 35 pounds of milk per day with a nutritive ratio of 1:8. This is an exceptionally "wide" ratio. Where the proteins were drawn from the corn or wheat kernels and constituted the same proportion of the corn stover roughage ration as indicated above, no such positive nitrogen balances could be maintained. We pointed out that during the negative nitrogen balance increased tissue autolysis resulted, and for a brief time at least there was no decrease in the milk proteins or milk solids elaborated.

In 1916[2] we showed that with *corn stover* and corn meal as the basal ration there were appreciable differences in the efficiency

* Published with the permission of the Director of the Wisconsin Agricultural Experiment Station.

[1] Hart, E. B., and Humphrey, G. C., *J. Biol. Chem.*, 1915, xxi, 239.

[2] Hart and Humphrey, *J. Biol. Chem.*, 1916, xxvi, 457.

with which certain protein concentrates could supplement such a ration for milk production. With the concentrates furnishing approximately 50 per cent of the total digestible proteins of the ration and the nutritive ratio fixed at approximately 1:8 (the total proteins constituted about 10 per cent of the dry matter of the ration) the gluten feed was measurably inferior to oil meal, distillers' grains, casein, or skim milk powder as a protein supplement in the particular mixture used. In the proportion used and with corn stover as the roughage none of these concentrates was able to maintain the animal in nitrogen equilibrium. A possible exception was milk powder. However, the distinctive point we wish to emphasize is that the negative nitrogen balance was much greater in the case of gluten feed than with the other materials used. The efficiency of the proteins of distillers' grains was attributed to the presence of the embryo of the seed.

We pointed out that the comparison would probably hold only for the mixture studied and that a different behavior might be expected should the basal ration be varied. Our expectations were entirely confirmed by the data to be presented. When the roughage of the ration was changed from corn stover to *clover hay* (medium red, *Trifolium pratense*) but the corn kernel maintained as the basal grain, the inefficiency of the gluten feed as a supplement disappeared, and among the four protein concentrates studied, namely, gluten feed, oil meal, distillers' grains (Ajax), and cottonseed meal there was little, if any, difference in efficiency. Furthermore, positive nitrogen balances were maintained during most of the periods of observation (16 weeks) on the nutritive ratio of 1:8.5.

EXPERIMENTAL.

The plan was to use a basal ration of clover hay, corn silage, corn meal, and starch, to which would be added the protein concentrate. The basal ration was maintained constant in relation to its source and proportion of nutrients for any individual in the different periods, the only variable in succeeding periods being the concentrate and the amount of starch. These were supplied in such quantities as to make the plane of protein intake and net available energy uniform in the several periods. After 16 weeks of observation with the various concentrates fed at a nutritive

plane of 1:8.5 the nutritive ratio was increased to approximately 1:5 for a period of 3 weeks by the addition of casein. This was done for the purpose of noting the effect of a high protein intake on both the quantity of milk secreted and its composition. This casein addition raised the total protein intake from approximately 12 per cent of the dry matter of the ration to 16 per cent.

Table I illustrates the proportion of the various feeds in the ration when an animal was receiving daily approximately 50 pounds of material.

TABLE I.

Source and Proportion of Nutrients Used.

Period	Gluten feed.	Distillers' grains.	Oil meal.	Cottonseed meal.
	lbs.	*lbs.*	*lbs.*	*lbs.*
Clover hay	8	8	8	8
Corn silage	28	28	28	28
Corn meal	6	6	6	6
Concentrate	4	3.37	3	2.88
Starch	4	4.63	5	5.12

Three Jersey cows of good milking capacity were used. Two were pure bred and one a grade. They were not with calf. The animals were milked twice daily and exercised two or three times a week. Their weight was taken weekly. The plan was to place each animal on any one of the rations for a period of 4 weeks with an immediate change to one of the other rations, thus involving each animal in 16 to 20 weeks of observation. A feeding period of 1 week preceded the quantitative collection of urine and feces.

The urine and feces were analyzed daily for nitrogen, while a weekly analysis was made of a 7 day composite sample of milk.

Our earlier observations had shown that when a nutritive ratio of 1:8 was used (equivalent to a plane of digestible protein intake of approximately 7 per cent, or 9 to 10 per cent of total protein) and the nutrients were drawn from corn stover, corn meal, and certain protein concentrates, a positive nitrogen balance could not be maintained. The daily production of milk in these earlier records was 35 to 40 pounds. Because of these facts it was planned to use rations with a nutritive ratio of 1:8 as it was essential that

TABLE II.

Composition of Rations.

	Weight.	Nitrogen.	Total N.	Production.	Digestible protein; nutritive ratio.
Gluten feed ration.					
	lbs.	*per cent*	*gm.*	*therms*	
Clover hay	8	1.90	69.00	2.75	
Corn silage	28	0.38	48.00	4.64	
Corn meal	6	1.65	44.90	5.38	2.2 lbs. digestible protein.
Gluten feed	4	3.98	70.20	3.17	
Starch	4	0.06	1.00	4.00	
Total	50		233.10	19.94	1:8.5
Distillers' grains (Ajax) ration.					
Clover hay	8	1.90	69.00	2.75	
Corn silage	28	0.38	48.00	4.64	
Corn meal	6	1.65	44.90	5.38	
Distillers' grains	3.37	4.65	71.40	2.66	
Starch	4.63	0.06	1.30	4.63	
Total	50		234.60	20.06	1:8.5
Oil meal ration.					
Clover hay	8	1.90	69.00	2.75	
Corn silage	28	0.38	48.00	4.64	
Corn meal	6	1.65	44.90	5.38	
Oil meal	3	5.25	71.50	2.36	
Starch	5	0.06	1.36	5.00	
Total	50		234.76	20.13	1:8.5
Cottonseed meal ration.					
Clover hay	8	1.90	69.00	2.75	
Corn silage	28	0.38	48.00	4.64	
Corn meal	6	1.65	44.90	5.38	
Cottonseed meal	2.88	5.47	71.44	2.18	
Starch	5.12	0.06	1.40	5.12	
Total	50		234.74	20.07	1:8.5
Oil meal-casein ration.					
Clover hay	8	1.90	69.00	2.75	
Corn silage	28	0.38	48.00	4.64	
Corn meal	6	1.65	44.90	5.38	
Oil meal	3	5.25	71.50	2.36	3.0 lbs. digestible protein.
Casein	1	12.71	57.65	1.00	
Starch	4	0.06	1.00	4.00	
Total	50		292.05	20.13	1:5

the animals be in negative nitrogen balance or just in nitrogen equilibrium in studies of this character. In the final adjustment of our rations the nutritive ratio became fixed at 1 : 8.5. In these rations this ratio was equivalent to approximately 8 per cent of digestible protein and 12 per cent of total protein. The composition of the rations used is shown in Table II.

TABLE III.

Record of Nitrogen Balance, Milk Nitrogen, Etc.

Animal 1, Jersey.

Date.	N intake.	N feces.	N absorbed.	N urine.	N milk.	N balance.
			Gluten feed period.			
	gm.	*gm.*	*gm.*	*gm.*	*gm.*	
Dec. 5–11	1,514	699	815	271	537	+ 7
" 12–18	1,452	678	774	292	545	− 63
" 19–25	1,435	633	802	266	536	00
" 26–Jan. 1	1,435	655	780	267	517	− 4
			Oil meal period.			
Jan. 2– 8	1,443	629	814	258	511	+ 45
" 9–15	1,443	591	852	233	524	+ 95
" 16–22	1,443	636	807	243	511	+ 53
" 23–29	1,443	680	763	242	523	− 2
			"Ajax" period.			
Jan. 30–Feb. 5	1,443	693	750	227	514	+ 9
Feb. 6–12	1,443	702	741	243	485	+ 13
" 13–19	1,443	718	725	208	454	+ 63
" 20–26	1,443	718	725	224	457	+ 44
			Cottonseed meal period.			
Feb. 27–Mar. 5	1,443	654	789	238	442	+107
Mar. 6–12	1,443	756	687	265	463	− 39
" 13–19	1,443	714	729	269	487	− 27
" 20–26	1,443	646	797	222	461	+114
			Oil meal-casein period.			
Mar. 27–Apr. 2	1,847				476	
Apr. 3– 9	1,847				521	
" 10–16	1,847				496	

TABLE IV.

Record of Nitrogen Balance, Milk Nitrogen, Etc.

Animal 2, Jersey.

Date.	N intake.	N feces.	N absorbed.	N urine.	N milk.	N balance.
			Gluten feed period.			
	gm.	*gm.*	*gm.*	*gm.*	*gm.*	
Dec. 5–11	1,076	462	614	296	295	+23
" 12–18	1,020	453	567	323	278	−34
" 19–25	978	431	547	294	269	−16
" 26–Jan. 1	978	475	503	285	245	−27
			Oil meal period.			
Jan. 2– 8	985	407	578	340	234	+ 4
" 9–15	985	396	589	265	244	+80
" 16–22	985	385	600	283	256	+61
" 23–29	985	385	600	273	267	+60
			"Ajax" period.			
Jan. 30–Feb. 5	985	515	460	230	255	−15
Feb. 6–12	985	442	543	214	245	+84
" 13–19	985	477	508	225	232	+51
" 20–26	985	500	485	226	260	− 1
			Cottonseed meal period.			
Feb. 27–Mar. 5	985	423	562	263	247	+52
Mar. 6–12	985	487	498	245	232	+21
" 13–19	985	484	501	230	240	+31
" 20–26	985	488	497	213	222	+62
			Oil meal-casein period.			
Mar. 27–Apr. 2	1,388				236	
Apr. 3– 9	1,388				254	
" 10–16	1,388				253	

The first four rations were much alike in content of production therms and total protein. Table II gives the composition of 50 pounds of the mixed ration. The animals were not fed 50 pounds daily, but what proportion of the ration would meet their maintenance and the energy requirements for their normal milk production. The production energy of the rations allowed con-

formed closely to Armsby's standard of 7 therms daily for maintenance and 0.3 therm for each pound of 4 per cent milk produced by a 1,000 pound cow. For example, Animal 1 weighed 950 pounds and produced daily 30 to 35 pounds of milk containing 4 to 5 per cent of fat, and consumed 44 pounds of the ration. In 50 pounds of the first four rations used there were approximately

TABLE V.

Record of Nitrogen Balance, Milk Nitrogen, Etc.

Animal 3, Jersey.

Date.	N intake.	N feces.	N absorbed.	N urine.	N Milk.	N balance.
			Oil meal period.			
	gm.	*gm.*	*gm.*	*gm.*	*gm.*	
Dec. 5–11	1,181	473	708	210	478	+ 20
" 12–18	1,181	567	614	220	467	− 73
" 19–25	1,181	509	672	182	465	+ 25
" 26–Jan. 1	1,181	548	633	169	455	+ 9
			Gluten feed period.			
Jan. 2– 8	1,181	477	704	207	453	+ 44
" 9–15	1,181	466	715	181	433	+101
" 16–22	1,181	455	726	193	441	+ 92
" 23–29	1,181	504	677	187	431	+ 59
			"Ajax" period.			
Jan. 30–Feb. 5	1,181	548	633	176	415	+ 42
Feb. 6–12	1,181	544	637	159	398	+ 80
" 13–19	1,181	556	625	158	400	+ 67
" 20–26	1,181	541	640	144	386	+110
			Cottonseed meal.			
Feb. 26–Mar. 5	1,181	484	597	193	370	+134
Mar. 6–12	1,181	659	522	197	381	− 56
" 13–19	1,181	539	642	193	382	+ 65
" 20–26	1,181	523	658	202	386	+ 70
			Oil meal-casein period.			
Mar. 27–Apr. 2	1,584				404	
Apr. 3– 9	1,584				396	
" 10–16	1,584				457	

2.22 pounds of digestible protein, 40 per cent of which came from the particular concentrate under investigation.

In Tables III, IV, and V are recorded by weekly periods the nitrogen balances and the nitrogen secreted in the milk. The figures represent the intake and output for the total 7 days.

In addition to the tables, charts are added showing the positive and negative nitrogen balances and the gm. of nitrogen produced in the milk. There are also added charts illustrating the volume of the milk secreted weekly and its content of solids and fat.

A survey of the data indicates a very uniform behavior in nitrogen metabolism with the different protein mixtures. There was no sudden or increased excretion of urinary nitrogen with the concentrate gluten feed as was observed in our earlier work where corn stover formed the roughage. Evidently the proteins of clover hay will supplement the proteins of gluten feed in a much more efficient manner than will those of corn stover. The same uniformity of nitrogen metabolism prevailed with the other concentrates. *These results emphasize in a very striking manner the limitations of any classification of natural foods in respect to the efficiency of their proteins, based on the determination of such nutritive work in a single food material or single mixture.* When they are used in mixtures as they generally are, the efficiency may be very greatly modified by the supplementary materials. This applies to human as well as animal nutrition. Lusk's[3] classification of the proteins of foods into the groups A, B, and C, according to their physiological value, has significance only in so far as the individual food materials form the sole article of diet. In a mixture, Class A would probably always improve Class C, but it is possible to conceive of Class C proteins from independent sources making an efficient mixture.

Positive nitrogen balances were maintained by all the animals over most of the periods of observation, although the plane of protein intake was low as compared with that usually prescribed for milking animals. These animals not only maintained nitrogen equilibrium, but also maintained their live weight. Their initial weights were 952, 762, and 683 pounds respectively. At the termination of the experiment they weighed 923, 800, and

[3] Lusk, G., Fundamental Basis of Nutrition, New Haven, 1914.

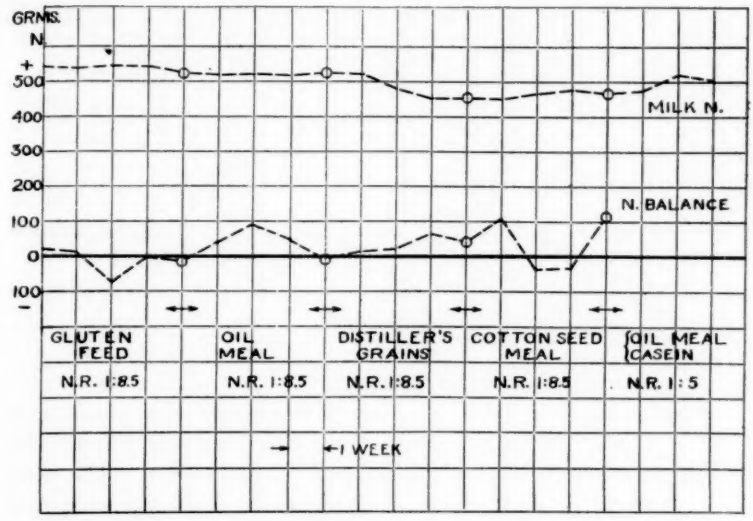

CHART 1. Animal 1. Showing the nitrogen balances with different sources of protein and the milk nitrogen production.

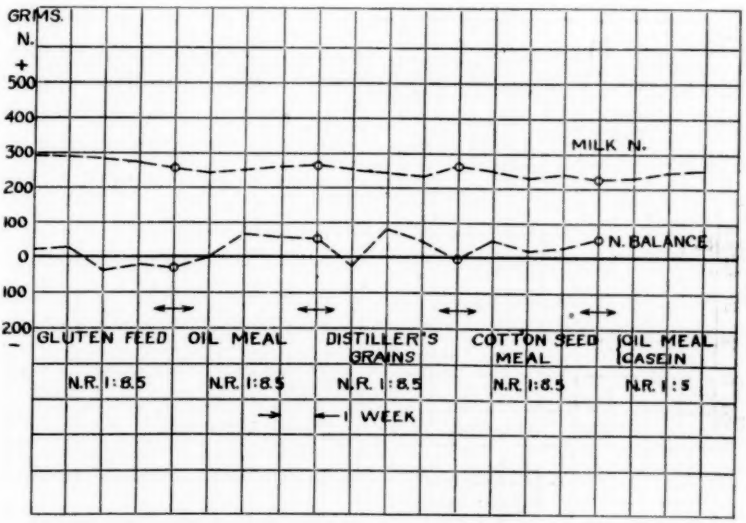

CHART 2. Animal 2. Showing the nitrogen balances with the different sources of protein and the milk nitrogen production.

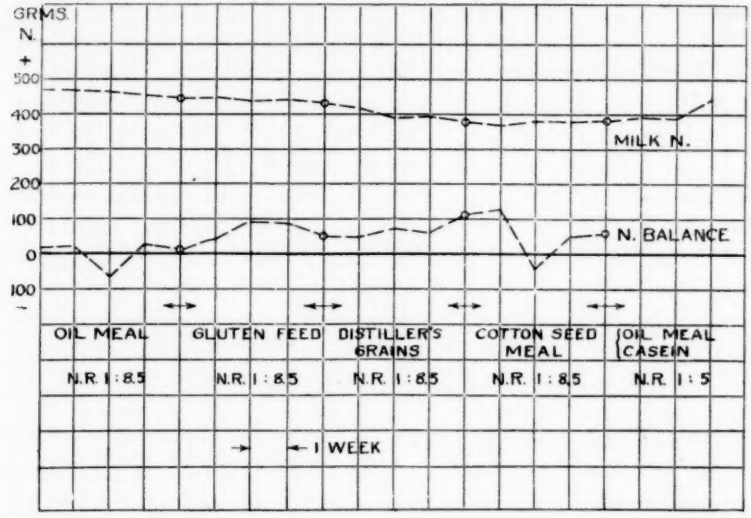

CHART 3. Animal 3. Showing the nitrogen balances with the different sources of protein and the milk nitrogen production. Attention should be called to the positive nitrogen balances maintained with gluten feed.

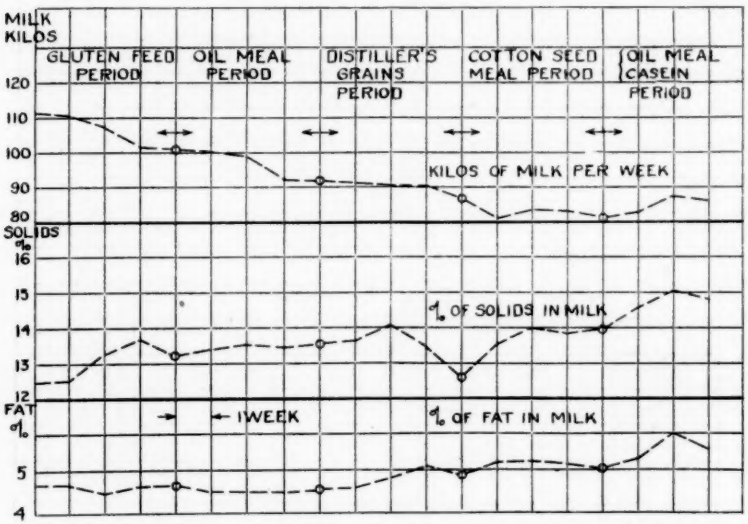

CHART 4. Animal 1. Showing the proportion of milk secreted and the solids and fat content of the milk.

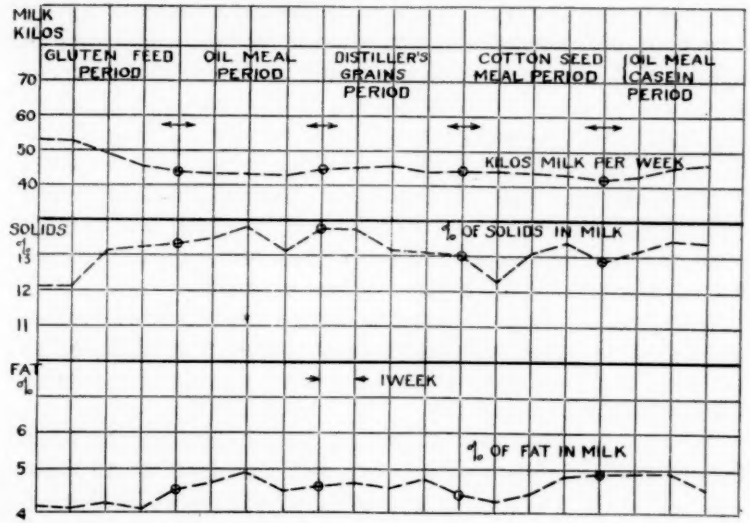

CHART 5. Animal 2. Showing the proportion of milk secreted and the solids and fat content of the milk.

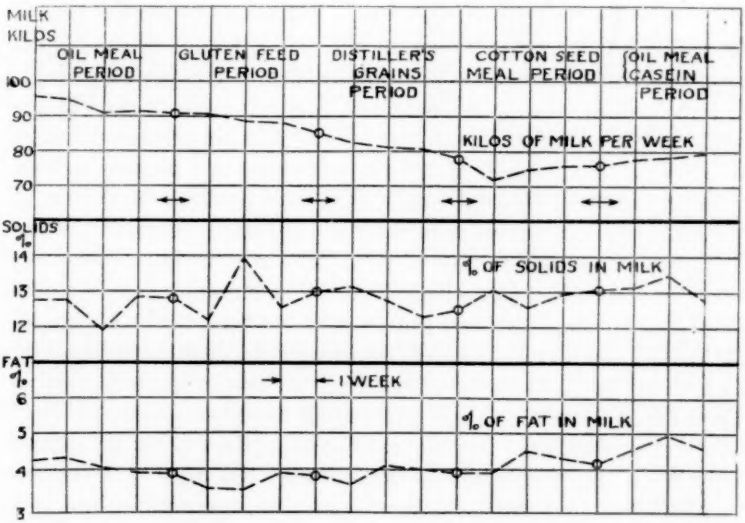

CHART 6. Animal 3. Showing the proportion of milk secreted and the solids and fat content of the milk.

689 pounds respectively. While all these animals were of strong dairy type and normally somewhat thin in flesh there was no evidence that they were more emaciated at the end of the experimental period than at its beginning. It is apparent that where ample net available energy is provided in a ration, a plane of protein intake may be found which will be lower than that prescribed by the standards and which will at least maintain nitrogen equilibrium, live weight, and a fairly well sustained flow of milk.

But the maintenance of nitrogen equilibrium, under such conditions, will depend upon the mixture of proteins used. Such nitrogen equilibrium as obtained in these experiments was impossible with any of the seed protein concentrates investigated when corn meal and *corn stover* formed the basal portion of the ration and were fed at a level of 1:8.

The mere maintenance of nitrogen equilibrium on the lower plane of protein intake and with selected materials did not, however, suffice for a sustained flow of milk. There was a gradual shrinkage in volume as the duration of the experiment progressed. The shrinkage was not large and was greater with some individuals than with others. Animal 1 was producing 35 pounds of milk daily when the experiment was initiated and 25 pounds before the period of high protein feeding; No. 2, 17 pounds at the beginning of the experiment and 13 pounds before the period of high protein feeding; No. 3, 31 pounds at the beginning of the experiment and 24 pounds before the period of high protein feeding. All showed a shrinkage in volume, but there was a maintenance of the percentage of total solids, fats, and proteins in the milk. But even with the maintenance of the percentage composition the shrinkage of volume means that there was actually a slow decrease in the elaboration of all classes of compounds by the mammary gland. In a previous publication[2] we presented data showing that a milch cow in negative nitrogen balance withdrew liberally from her own tissues, for a time at least, and continued to elaborate milk proteins; but under such conditions there was not only a slow shrinkage in volume of milk produced, but an actual decrease in the percentage composition of the milk. In the experiment reported in this paper where nitrogen equilibrium was maintained, but the protein level of intake was low, shrinkage of flow followed, but the percentage composition was maintained.

To show these effects the composition and volume of the milk secreted by the several animals at different periods of observation are given in Table VI.

TABLE VI.

Decrease in Milk Volume, but Maintenance of Percentage Composition on Low Protein Intake, but in Nitrogen Equilibrium.

Animal No.		Dec. 15.	Jan. 12.	Feb. 9.	Mar. 9.	High protein, Apr. 6.
1	Total solids, *per cent*	13.40	15.60	14.10	14.00	15.10
	Fat, *per cent*	4.30	4.40	4.80	5.30	6.00
	Nitrogen, *per cent*	0.51	0.51	0.53	0.53	0.57
	Milk daily, *lbs*	33.60	31.70	28.80	27.10	28.20
2	Total solids, *per cent*	13.30	15.90	13.20	13.30	13.50
	Fat, *per cent*	4.20	4.90	4.50	4.40	4.90
	Nitrogen, *per cent*	0.57	0.51	0.54	0.56	0.54
	Milk daily, *lbs*	15.00	14.40	14.70	13.90	14.50
3	Total solids, *per cent*	11.90	14.00	12.70	12.60	13.50
	Fat, *per cent*	4.00	3.90	3.90	4.40	4.50
	Nitrogen, *per cent*	0.50	0.46	0.47	0.47	0.51
	Milk daily, *lbs*	28.20	27.00	25.30	24.30	23.00

In the period of high protein feeding there was not only a stimulation to flow, but an actual increase in solids secreted. The peculiar stimulating effect of liberal protein feeding on mammary activity was strikingly shown in these records. The maintenance of milk flow desired by every dairyman is very probably secured by his customary high protein feeding, but at what expense is not so clear. Whether we could have partly prevented the shrinkage of milk flow observed in these experiments by the use of better or higher protein intake cannot be answered, but in practice where a high protein level is used there is also a decreased flow of milk incident to an advancing lactation. In view of the fact that there was an acceleration of mammary gland activity during the period of high protein feeding it is probably correct to attribute some of the milk shrinkage observed in this work to a low protein intake. From the data in Table VII it should be noted that if we include the tissue nitrogen storage with that of the milk there was not an appreciable decrease in nitrogen utili-

zation for the combined process of milk production and tissue reparation with advancing lactation. A redistribution of the nitrogen between milk and tissue was slowly in progress.

Efficiency of Protein Mixtures Compared.

The basis for comparing the efficiency of these protein mixtures involves not only the milk proteins elaborated, but in addition the protein catabolized or stored during the periods of observation. We had expected that our animals would be in negative nitrogen balance on the low protein level used, but for most of the periods positive nitrogen balances prevailed. The fact that the storage of nitrogen was in any case but slight while the flesh condition of the animals would probably have allowed more ample protein storage had it been available makes it evident that we were in all cases approximately close to nitrogen equilibrium. While it is necessary that negative nitrogen balances prevail for measuring the efficiency of the protein mixture for milk production, particularly where the animal is in good flesh, yet it is possible to make accurate measurements of this efficiency if the animal is in positive nitrogen balance, but in poor flesh. This then involves the protein in both milk production and tissue building. For the reason that our animals were rather thin in musculature and that positive nitrogen balances were at most but slight we have confidence in the results recorded. For purposes of making definite comparisons we have calculated the percentage of efficiency for the various mixtures used on the basis of absorbed nitrogen, tissue destroyed or stored, and milk proteins produced. Manifestly the absorbed nitrogen should be used in the calculation rather than the total nitrogen ingested. In Table VII these comparisons are made.

The average percentage of efficiency was very uniform with the different concentrates and the same individual. Much the greatest variation was among the individuals. Chart 7 illustrates these differences. Animal 2 showed an *average* efficiency on all protein mixtures of but 51 per cent, with the lowest on gluten feed (46 per cent) and the highest on distillers' grains (55 per cent); while No. 3 showed on the same rations on *average* efficiency of 72 per cent, with the highest efficiency (75 per cent) on distillers' grains and the lowest (70 per cent) on oil meal.

TABLE VII.

Relative Efficiency for Milk Production of Protein Mixtures Involving Gluten Feed, Distillers' Grains, Oil Meal, and Cottonseed Meal.

Animal No.	Date.	Ration.	N absorbed.	N in milk ± tissue N formed or destroyed.	Efficiency.
			gm.	*gm.*	*per cent*
1	Dec. 5–Jan. 1	Gluten feed.	3,171	2,075	65
	Jan. 2–29	Oil meal.	3,236	2,260	69
	" 30–Feb. 26	Distillers' grains.	2,941	2,039	69
	Feb. 27–Mar. 26	Cottonseed meal.	3,002	2,008	66
2	Dec. 5–Jan. 1	Gluten feed.	2,231	1,033	46
	Jan. 2–29	Oil meal.	2,367	1,205	50
	" 30–Feb. 26	Distillers' grains.	1,996	1,111	55
	Feb. 27–Mar. 26	Cottonseed meal.	2,058	1,107	53
3	Dec. 5–Jan. 1	Oil meal.	2,627	1,846	70
	Jan. 2–29	Gluten feed.	2,822	2,054	72
	" 30–Feb. 26	Distillers' grains.	2,535	1,898	75
	Feb. 27–Mar. 26	Cottonseed meal.	2,419	1,732	71

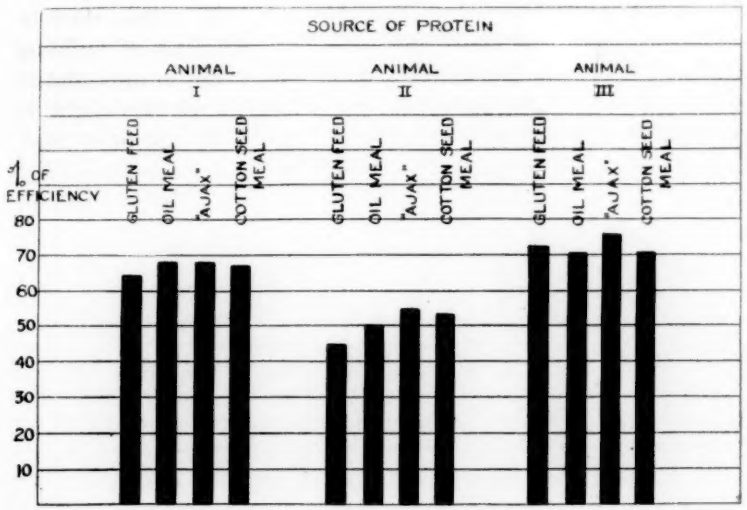

CHART 7. Showing the comparative efficiency for milk production of the different protein mixtures. Note the uniformity of any one animal with the different mixtures, but the large individual variation.

SUMMARY.

Data are presented on the comparative value for milk production of protein mixtures involving gluten feed, oil meal, distillers' grains, and cottonseed meal.

These concentrates furnished approximately 40 per cent of the digestible protein of the ration and were used to supplement a basal ration of corn meal, corn silage, and clover hay. The total protein intake constituted about 12 per cent of the dry matter of the ration and the nutritive ratio was approximately 1: 8.5.

On this low protein intake positive nitrogen balances were maintained during most of the period of observation (16 weeks) with a slow shrinkage in milk volume, but a maintenance of the percentage composition of the milk.

Earlier records showed the inferiority of the proteins of gluten feed as a supplement to the proteins of corn meal and *corn stover* for milk production to those of oil meal, distillers' grains, or milk. These records show an equality in efficiency between the proteins of gluten feed, oil meal, distillers' grains, and cottonseed meal as supplements to the proteins of corn meal and *clover hay* for milk production.

These facts must emphasize in a very striking manner the limitations of any classification of natural foods in respect to the efficiency of their proteins, based on the determination of such nutritive worth in a single food material or a single food mixture.

A STUDY OF THE EFFECT OF HYDROCHLORIC ACID ON THE MINERAL EXCRETION OF DOGS.*

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(Received for publication, June 22, 1917.)

It is conceivable that the administration of an unoxidizable acid to a normal living organism or the formation of such an acid by the organism itself may exert a deleterious action (1) by neutralizing alkali necessary for the transportation of carbon dioxide formed in the metabolic processes; (2) by direct toxic action or by the toxic action of a resulting salt; (3) by diminishing tissue alkalinity and thereby influencing metabolic activities (this change may be in the potential alkalinity rather than in the actual hydroxyl ion concentration, the effect then being due to a diminution in the cation concentration below that which is essential for normal metabolism). To determine which of these three possibilities actually occurs has been the basis for numerous investigations, the ultimate object being to explain the coma of diabetes mellitus. Experimental evidence has been obtained in support of each.

HISTORICAL.

To determine whether the body is depleted of its stock of alkali necessary for the transportation of carbon dioxide several methods have been employed. They have been direct and indirect, the latter type predominating. Where the direct method—the quantitative determination of the bases excreted—has been employed it has not been carried out in detail, that is, the bases have not been determined individually. Carnivora and

* The work here reported was undertaken jointly with Dr. A. E. Taylor but the utilization of the latter's time by the national government has made his participation impossible. The author desires to express his thanks to Dr. Taylor, however, for placing his own previous experience in connection with the problem at the writer's disposal.

herbivora have been used and the results seem to indicate that much depends upon the dietary habits of the experimental animals. Salkowski (1873), using rabbits and feeding taurine with the expectation that it would be oxidized to sulfuric acid, observed an increase in both the sodium and potassium outputs. He was led by the variation of his own results from the earlier negative results of Gaethgens (1872), Eylandt (1854), and Wilde (1855), obtained with dogs and men, to postulate a difference between carnivora and herbivora in the matter of alkali excretion after acid administration. Salkowski did not determine the ammonia excreted and it remained for Walther (1877) to discover that in the dog the acid was excreted to a great extent as the ammonium salt. For this reason Walther believed dogs to be much more resistant to acids than rabbits whose death he explained as due to the withdrawal of alkali necessary for the transportation of carbon dioxide. Walther's figures showing an enormous decrease in the carbon dioxide content of the blood give credence to this belief. Eppinger (1906) has reported experiments intended to settle the question of a difference between carnivora and herbivora as regards resistance to acid. His results were such as to justify the conclusion that rabbits ordinarily react differently from dogs merely because their diet is low in protein. By proper protein feeding he was able to increase the resistance of rabbits to acid and by administering amino-acids or urea simultaneously with the acid he found that the symptoms of coma did not appear. Pohl and Münzer (1906), Pohl (1909), and Bostock (1913) dispute the correctness of Eppinger's results though Eppinger and Tedesko (1909) have repeated and extended the former's experiments. The names of Auerbach (1884), Winterberg (1898), Kettner (1902), Spiro (1902), and Labbé and Violle (1911) should be mentioned in connection with this phase of acid intoxication studies.

Of the direct methods for determining the effect of acid administration upon the withdrawal of alkalies the determination of the carbon dioxide content of the blood and of the latter's capacity to neutralize acid have been employed most frequently. Parallel analyses have been made by many investigators. The results of most of the experiments reported show that in acid intoxication both carbon dioxide content and titratable alkalinity are lowered. Lassar (1874), Kraus (1889, 1890), Loewy and Münzer (1901), Spiro (1902), and Landau (1905) have helped develop this aspect of the problem.

Low carbon dioxide values are by no means confined to acid intoxication. They have been found to follow the administration of phosphorus, iron, arsenic, emetin, and many other substances (Meyer and collaborators, 1881, 1883), in fever (Kraus, 1889), after the administration of sodium butyrate and sodium isobutyrate (Loewy and Erhmann, 1911), etc. In diabetes Beddard, Pembrey, and Spriggs (1904) found the carbon dioxide content and acid neutralizing power diminished. They found also, however, that the blood had not lost its power to bind carbon dioxide. By tying off the arm of a diabetic they observed the carbon dioxide content to be increased from 40 to 45 volumes per cent to 61.8 volumes per cent. The low carbon

dioxide values are attributed by them to a stimulation of the respiratory center by acids other than carbonic and to a diminished carbon dioxide production.

Some attempt has been made to measure the effect of acid on the blood alkali by measuring the hydrogen ion concentration after acid administration and by a study of the blood of diabetics. Szili (1906), by intravenous injections of acids into dogs, found the hydrogen ion concentration decreased. Benedict (1906), Rolly (1912), and Poulton (1916) found the hydrogen ion concentration increased in some diabetics and unchanged in others.

The significance of a low carbon dioxide content of the blood is detracted from by the results of Beddard, Pembrey, and Spriggs, already mentioned. Data given by Benedict and Joslin (1910) do not support the low alkali theory of diabetic coma any better. These authors found the total metabolism of diabetics to be somewhat higher than that of normal individuals but their data show respiration and pulse rate to be at least as low as normal, which would indicate that the body is experiencing no difficulty in eliminating its carbon dioxide. If the hydrogen ion concentration is the controlling factor in respiration a deficiency of alkali should surely show itself by the action of the increased amount of carbon dioxide in solution on the delicate mechanism which the respiratory center is supposed to be. The damming up of carbon dioxide in the tissues should also show itself in an increase in the carbon dioxide content of the urine in severe diabetes but such does not occur according to Beddard, Pembrey, and Spriggs.

The hypothesis that diabetic coma is attributable to toxic action has found some supporters. It is sufficient here to call attention to the work of Mayer (1886), Wilbur (1904), Marx (1910), and Ehrmann and his collaborators (1911).

Inasmuch as there are no complete data on the excretion of the metallic elements during acid intoxication the third hypothesis referred to above has little to support it. Chvostek (1893) found the oxygen utilization, carbon dioxide production, and heat formation lowered during acid intoxication. In agreement with these results are some older experiments of Munk (1881) which showed that after long treatment with hydrochloric acid the high oxidizing capacity of the horse for phenol was reduced 41.2 per cent. The results of the present investigation furnish evidence for believing that diabetic coma may *possibly* be a result of the withdrawal of essential cations.

EXPERIMENTAL.

No detailed study has been made, as mentioned above, of the excretion of bases after acid administration. It seemed desirable, therefore, to subject this phase of the problem of acid intoxication to a fuller investigation.

A female dog weighing between 9 and 10 kg. served for the two experiments to be reported here. The diet consisted of 50 gm. of soda crackers, 150 gm. of beef heart, and 10 gm. of agar-agar intimately mixed and containing carmine every other day. The acid, in the first period of the series was given in the form of glycocoll hydrochloride (enclosed in balls of food) and in the second period as dilute hydrochloric acid (0.125 N and 0.250 N). When dilute acid was employed the same volume of water was given on the control days. No difficulty was experienced by the animals in retaining the amounts administered. Chlorinated fat was tried with the hope that by its use large doses of potential hydrochloric acid might be given in small volume but the material employed which contained 21 per cent chlorine was not retained by the animals. Very small quantities were vomited. 24 hour samples of urine were collected by catheterization at the end of each experimental day, that portion which was voided spontaneously being collected over chloroform. The feces were separated as nearly as possible into portions corresponding to the food from which they came but the analytical results indicate that such a procedure does not give as clear a picture of the excretion of bases as is obtainable from the urine. Average figures for the various periods are more significant in the case of the feces.

Analytical Methods.

Urine and feces were ashed in the wet way and the solution then evaporated to dryness. For determining the sodium and potassium the residue was heated with dilute hydrochloric acid and the solution then precipitated with saturated barium hydroxide solution until the reaction was alkaline. The precipitate was separated with the aid of the centrifuge and the excess barium precipitated with sulfuric acid. The filtrate on evaporation gave combined sodium and potassium sulfates. (In the case of feces the sulfuric acid solution contained some calcium which was removed by precipitation in ammoniacal solution with ammonium oxalate.) Potassium alone was then estimated by precipitating it as potassium sodium cobaltic nitrite and titrating the nitrous acid with standard permanganate. The details of the method are described by Drushel (1908). Sodium was determined by

difference. The procedure for calcium and magnesium was the ordinary oxalate and pyrophosphate method. In the case of urine known amounts of calcium and magnesium were previously added in order to be better able to judge of the satisfactoriness of the precipitation.

DISCUSSION.

Chart I shows the variations in the potassium excretion during the 26 days of the experiment. The normal potassium excretion on the diet employed is close to 400 mg. per day. The administration of 9 gm. of glycocoll hydrochloride caused the output to rise immediately, but it is noteworthy that even while the hydro-

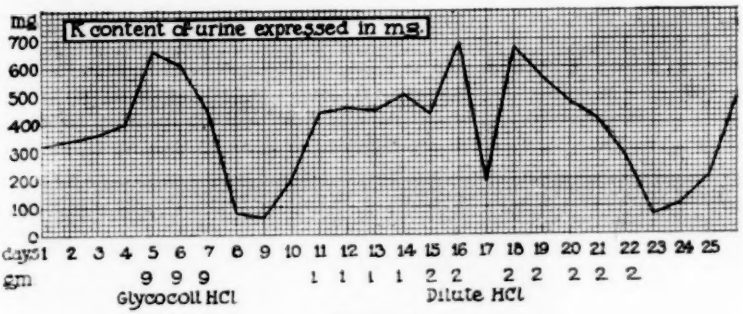

CHART I.

chloride was still being administered the excretion began to fall and on the 8th day when none was given the excretion was only one-fourth of the normal amount. On the 10th day the rate of excretion was again approaching the normal but rose noticeably on subsequent days when free hydrochloric acid was given. The same subnormal excretion, when the administration of acid was stopped, and even before, is again apparent. It would appear as though the experience of a day or two enabled the animal to combat successfully the attempt to withdraw its potassium supply and even to recover by subnormal excretion what was lost originally by the administration of acid.

Chart II, showing the sodium excretion, is less clear. The maxima of the curves parallel those of the potassium excretion,

however, and it is probably true that the same phenomenon occurs here though to a less pronounced degree.

These results are difficult to reconcile with the conclusions based upon the determinations of blood carbon dioxide and titratable alkalinity. The results of such determinations seem to require a loss of alkali but evidence of its elimination does not appear here.

The calcium and magnesium contents of the urine are almost insignificant, say 20 mg. of each on the diet employed. Chart

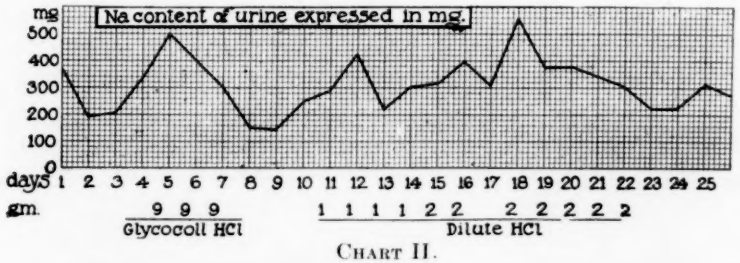

CHART II.

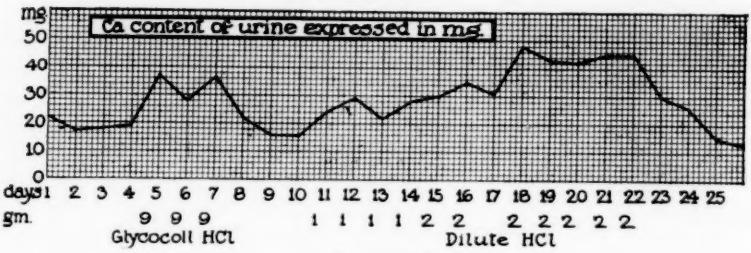

CHART III.

III shows, however, that the same variations occur here as in the case of potassium with possibly a less pronounced tendency to recover lost base after the administration of acid has ceased. The magnesium excretion, Chart IV, bears the same relation to the calcium excretion that the sodium excretion does to the potassium. It can hardly be denied that there is a slight tendency for the magnesium excretion to increase during the administration of acid.

The results of the urine analyses are shown collectively in Chart V. Here the quantities are plotted in gm. equivalents ×

10,000 instead of in mg., with the result that the relative rôles in neutralizing the acid administered are made apparent. It may be seen that when so plotted the variations in the calcium and magnesium excretions are hardly perceptible. The variations in

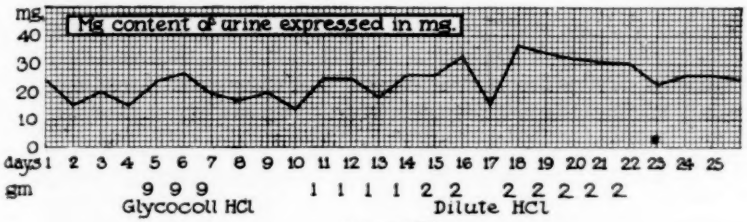

CHART IV.

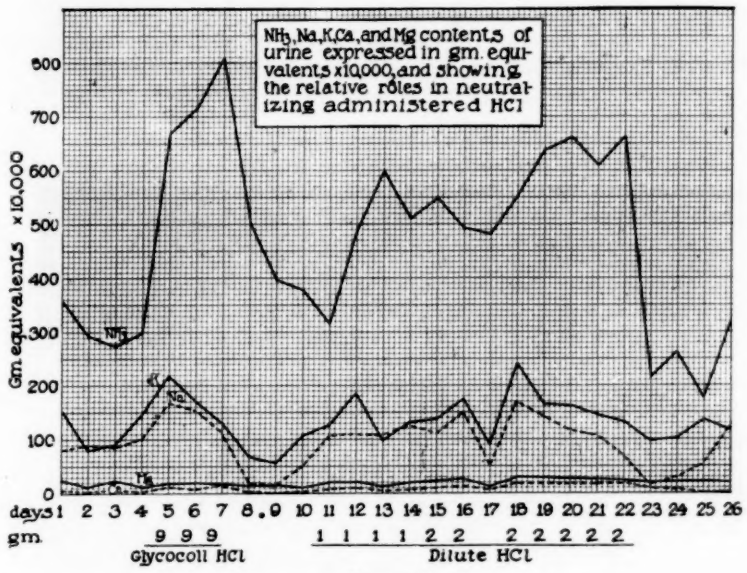
NH3, Na, K, Ca, and Mg contents of urine expressed in gm. equivalents x10,000, and showing the relative rôles in neutralizing administered HCl
Gm. equivalents x10,000
800
700
600
500
400
300
200
100
0
NH3
K
Na
Ca
Mg
days 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26
gm 9 9 9 1 1 1 1 2 2 2 2 2 2 2 2
Glycocoll HCl
Dilute HCl

CHART V.

the potassium and sodium outputs are plainly in evidence but they are small in comparison with the changes in the ammonia output. This base is the great factor in neutralizing the acid administered.

As was stated above, the analysis of the feces corresponding to the food of each of the 26 days of the experiment does not give results which make their plotting individually very significant. However, it is not to be expected that the feces corresponding to the food of a day on which acid was given will necessarily reflect the effect of that acid. It may be that the excess of base excreted will appear in the feces corresponding to the food of an earlier day and indeed some of the results seem to indicate as much. In addition it was impossible to separate the feces sharply even when marked as they were and some of the fluctuations are attributable to this fact. By taking average excretions of the

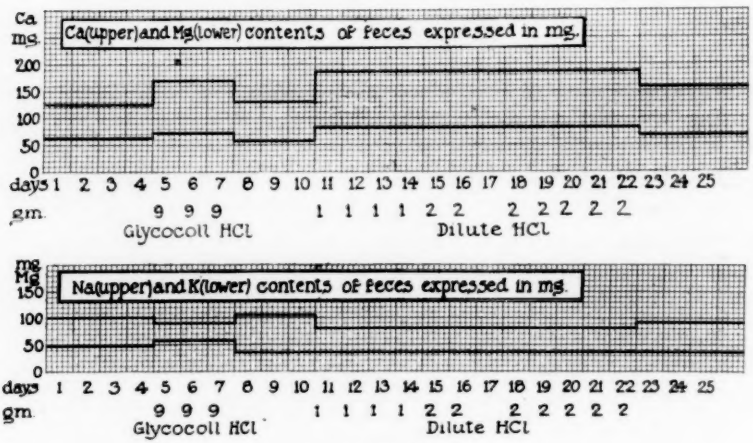
Ca
mg.
Ca(upper) and Mg(lower) contents of feces expressed in mg.
200
150
100
50
0
days 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25
gm. 9 9 9 1 1 1 1 2 2 2 2 2 2 2
Glycocoll HCl
Dilute HCl
mg
Mg
Na(upper) and K(lower) contents of feces expressed in mg.
150
100
50
0
days 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25
gm. 9 9 9 1 1 1 1 2 2 2 2 2 2 2
Glycocoll HCl
Dilute HCl

CHART VI.

different periods, however, figures are obtained which when plotted give Chart VI. There can be no doubt that both the calcium and magnesium excretions are increased to a greater extent than in the urine. This is not true of the sodium and potassium, and it is a fair conclusion that the results in this case do not indicate any loss of these two elements through the intestine during acid administration. It may be that a more detailed study would show an initial rise followed by a compensating fall.

It seems right to conclude from the results obtained that calcium and to a less extent magnesium are the only metals lost by acid administration. Taking the normal calcium excretion in the

present case as 130 to 140 mg. per day in the feces and 20 mg. in the urine, a calculation shows that the administration of 2 gm. of hydrochloric acid causes an additional excretion of about 75 mg. Now 1 gm. of hydrochloric acid is equivalent to 2.85 gm. of β-hydroxybutyric acid, and if we consider a 70 kg. man instead of a 10 kg. dog, the corresponding β-hydroxybutyric acid excretion would be 40 gm. This is close to the maximum excreted in severe diabetes. When such a quantity of this acid is being eliminated the loss of calcium would amount to 0.53 gm. In one of the cases of diabetes reported by Benedict and Joslin (1910) β-hydroxybutyric acid determinations were made frequently and in the last 160 days of the patient's life calculation shows that somewhat more than 5 kg. of β-hydroxybutyric acid were excreted. Inasmuch as it was just found that 40 gm. of the acid corresponds to 0.53 gm. of calcium, the loss of this element during the 160 days would amount approximately to 66 gm. Such an amount does not seem large when the whole calcium content of the body is considered but it is unknown, of course, to what extent the calcium of the body must be considered as inert. If the quantity is large then 66 gm. may be quite a significant loss. The analogy between man and the dog may not be complete and perhaps is not. It is likely, for example, that because of man's lower protein diet ammonia may play a less important rôle in neutralizing ammonia than it does in the dog, with the result that the loss of other cations would be augmented and the harmful effects increased.

CONCLUSIONS.

The administration of hydrochloric acid by mouth to the dog causes an increased excretion of calcium and magnesium as well as of sodium and potassium but in the case of the latter pair a compensatory retention makes the loss apparent rather than real.

If an analogous condition holds in human diabetes the resulting calcium loss may be something to take into consideration in the treatment of diabetic patients in whom the excretion of hydroxybutyric acid has reached a significant figure.

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THE RELATION OF ADRENALIN HYPERGLYCEMIA TO DECREASED ALKALI RESERVE OF THE BLOOD.

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During the course of some attempts by one of us to increase the food tolerance of diabetic patients by subcutaneous injections of adrenalin, it was observed that one of the patients showed more or less pronounced hyperpnea immediately after the administration of adrenalin. This observation, taken in connection with the fact that lowering of the alkaline reserve of the blood is often accompanied by some degree of hyperpnea, led to the investigation of the effect of adrenalin upon the CO_2 combining capacity of the blood. The fact that adrenalin also causes hyperglycemia, that the administration of acid increases this hyperglycemia (1), and as pointed out by Elias (2), that the intravenous injection of acid alone causes hyperglycemia, led to the suspicion that adrenalin causes hyperglycemia by lowering the alkaline reserve of the blood. Therefore to investigate this hypothesis parallel estimations of blood sugar and blood CO_2 combining capacity have been made after administration of adrenalin in cases of diabetes and in normal individuals.

To complete the study, observations on alveolar CO_2 tension, blood pressure, pulse rate, and glycosuria are added.

Three cases of diabetes were chosen because of the ease in which rapid and extreme changes in blood sugar can be produced. Later two normal men were also studied in the same way, showing that the changes observed were not peculiar to diabetics.

The procedure was as follows: All the cases were studied fasting, the periods of fasting before the administration of adrenalin ranging from 11 to 41 hours. From 15 to 30 minutes before the administration of adrenalin and at varying intervals thereafter, blood was obtained for estimation of the plasma CO_2 combining

capacity and for sugar analysis. Samples of alveolar air were taken for estimation of the CO_2 tension. Blood pressure and pulse rate were also determined.

Adrenalin 1: 1,000 (Parke, Davis and Company) was given subcutaneously in doses of 10 to 21 mg. Patients were carefully watched for appearance of symptoms, and the fast was continued to the end of the period of observation.

The blood sugar was determined by the method of Lewis and Benedict and the blood CO_2 by the Van Slyke method, the results being expressed in terms of alveolar air. Alveolar CO_2 tension was determined by the Fridericia method. The sugar in the urine was determined quantitatively and qualitatively by the Benedict methods. Charts and protocols follow at the end of the paper.

From a study of the charts it will be noted that in all the experiments the subcutaneous administration of adrenalin in doses ranging from 10 to 21 mg of the 1: 1,000 solution produced a diminution in the blood CO_2 combining capacity synchronous with a rise in the blood sugar concentration. These changes occurred in all cases in varying degrees within the first half hour and reached their maximum intensity within 3 hours. They were followed within 6 to 8 hours by a drop in the blood sugar to its original level or to a level lower than that previously observed. The blood CO_2 combining capacity returned to its original level or above except in Cases 3 and 4, where within the experimental period the former level was not quite attained. We have not been able to explain this observation. In Case 5 the blood CO_2 returned to approximately its original level within the 1st hour but 4 hours later it was even higher than it had been in the first observation after adrenalin. In this case it will be observed that a relatively small dose of adrenalin was given, the reaction in every respect was comparatively slight, and as in Case 1, there was no sugar in the urine.

Alveolar CO_2.—The incompleteness of the observations as regards the alveolar CO_2 is due to the difficulty encountered in getting good samples of air in Cases 1 and 2, while in Case 3 the same difficulty obtained half an hour after adrenalin was given.

DISCUSSION.

It might be argued that the hyperpnea produced by adrenalin was not due to a change in the reaction of the blood and that the low values obtained for the plasma carbonates were due merely to a diminution of the CO_2 tension of the blood caused by over ventilation. This would reduce the whole effect to an expression of the Zuntz reaction, a shifting of the carbonates from the plasma to the cells in response to a lowering of the carbon dioxide concentration of the blood. If this were the case the alveolar air determination should reveal the effects of the "*Auspumpung.*" The alveolar values, however, are higher than those obtained from the plasma, an indication that the respiratory mechanism has not been able to remove the increased CO_2 offered to it (3, 4). On the other hand, if we were dealing with a simple carbon dioxide acidosis the plasma carbonates should rise instead of fall. The dyspnea and the fall in plasma carbonates must, therefore, represent a real diminution of the fixed alkali of the blood.

The mechanism of epinephrin hyperglycemia and glycosuria, although not yet clear in all its details, can be ascribed most probably to an increase in glycogenolysis. The evidence has been thoroughly discussed in a recent paper by Mackenzie (5) and need be considered here only as it has a bearing on our special problem. The effects of epinephrin, especially as regards carbohydrate metabolism, are strikingly like those of acid though very little attention has been paid hitherto to this aspect of its behavior.

Elias (2) was the first to point out that the administration of acid produced a hyperglycemia and glycosuria and that this was due to an increased glycogenolysis. This action was independent of the adrenals and could be demonstrated even in the perfused liver washed free from blood. Alkali produced an opposite effect. He used hydrochloric acid and sodium carbonate. Macleod (6) found that the hyperglycemia and glycosuria of asphyxia were dependent on a carbon dioxide acidosis. Both the hyperglycemia and glycosuria failed to appear when the liver was excluded from the circulation, but were not prevented when the hepatic nerve plexus was cut. The parallelism between the action of acid and the action of adrenalin fails only inasmuch as the investigation on the effect of acid is not so complete as the investigation on the effect of adrenalin.

Underhill (1) was able to increase epinephrin hyperglycemia and glycosuria by the administration of hydrochloric acid, and, what is more significant, he was able to diminish hyperglycemia and glycosuria and even, in one case, to prevent it by the administration of sodium carbonate. The inhibitory effect of carbonate in preventing hyperglycemia and glycosuria was obtained only when it was injected at least half an hour before the epinephrin. This suggests that carbonate acts by establishing conditions unfavorable to the production of the regular adrenalin effect.

In view of this experimental evidence and the time relations of the CO_2 and hyperglycemia curves, it seems more than probable that at least a part of the hyperglycemia and glycosuria following the injection of adrenalin was caused by a diminution of the alkalinity of the blood.

Diminished blood alkalinity as the probable cause of hyperglycemia is particularly suggested by a study of the time relation of the curves in the three diabetic cases (1, 2, and 3). In the charts of these cases it will be noticed that the apex of the blood CO_2 curve is attained from 1 hour to 1 hour and 40 minutes before the apex of the blood sugar curve is reached. This did not occur in the two normal individuals whose blood sugar and blood CO_2 curves reached their peak simultaneously. A possible explanation of this discrepancy lies in the fact that in the diabetic the whole reaction extends over a longer period than in the normal cases. In this connection it is possible that determinations made sooner after the administration of adrenalin and at more frequent intervals in the normal cases would have shown a similar relationship.

We have recognized that the change in blood reaction and change in sugar content have occurred in all cases at the time of the first observation, that is, within 20 minutes. Whether it could be found that the changes occurred simultaneously and immediately after adrenalin was given or whether one precedes the other has not been shown.

Determinations at 2 to 5 minute intervals after adrenalin might possibly show that the blood CO_2 change preceded the blood sugar change, and if that were the case the evidence would be even more convincing that diminished alkalinity played the lead-

ing rôle in the production of hyperglycemia. It was difficult, however, to find patients willing to submit to such frequent vena puncture as this plan would necessitate and such estimations were not made.

The degree of acidemia which was produced by the injection of adrenalin was probably sufficient to bring about the increase in the blood sugar, for Elias by the injection of acids was able to induce glycosuria in dogs even when the acidemia was not sufficient to cause air hunger. In two of our cases the acidemia following adrenalin was accompanied by severe hyperpnea. That this hyperpnea was not out of proportion to the change of blood reaction was evident from the fact that it was not sufficient to lower the alveolar CO_2 tension to the level required by the concentration of carbonates in the plasma.

Ritzmann (7), who administered adrenalin intravenously, studied its parallel effect upon glycosuria and hypertension. He concluded that adrenalin affected carbohydrate metabolism only when it caused vasoconstriction. He did not make observations on the blood sugar.

Lusk (8) found that adrenalin was without influence upon the oxidation of sugar and agreed with Ritzmann that adrenalin acted upon carbohydrate metabolism by vasoconstrictor effect, the vasoconstriction causing asphyxia of the tissues. Pollak (9) showed that both hyperglycemia and glycosuria were more readily produced by subcutaneous than by intravenous administration of adrenalin. It is well known that vasoconstrictor effects are uncertain after subcutaneous administration. In two of our cases after the subcutaneous administration of adrenalin the rise in the blood sugar was accompanied by hypertension. In two others there was an increase in the blood sugar but no rise in the blood pressure. This does not rule out vasoconstriction of the liver vessels but a vasoconstriction sufficient to cause asphyxia of the tissues is at least improbable. Tissue asphyxia, however, in itself increases the acidity of the blood and tissues.

Epinephrin and acid also have other physiological properties in common. According to Trendelenburg (10) they both cause relaxation of the bronchial muscle and vasoconstriction. How far the parallelism can be carried and to what extent the action of adrenalin is dependent upon the associated acidosis it is impossible to say.

The discovery of an acid intoxication from adrenalin is strangely at variance with Crile's theory of shock, but the experimental evidence of an alkalinizing action on the part of the adrenals brought forward by Menten and Crile (11) is entirely unsatisfactory. Bedford (12) has recently shown that, contrary to Crile's statement, there is an increase in the adrenalin content of the blood flowing from the adrenals, during prolonged shock in dogs.

CONCLUSIONS.

1. The hyperglycemia produced by subcutaneous injection of adrenalin in three cases of diabetes and two normal individuals was accompanied by simultaneous diminution of the alkalinity of the blood. This taken in conjunction with other experimental evidence strongly suggests that decreased alkalinity of the blood plays a very important rôle in the production of hyperglycemia of this type.

2. Vasoconstriction as demonstrated by peripheral hypertension is not of prime importance in producing the changes noted.

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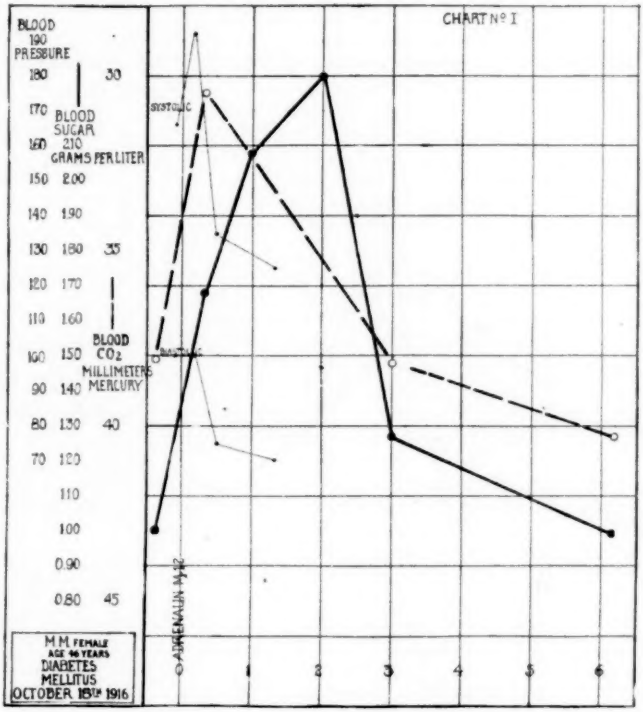
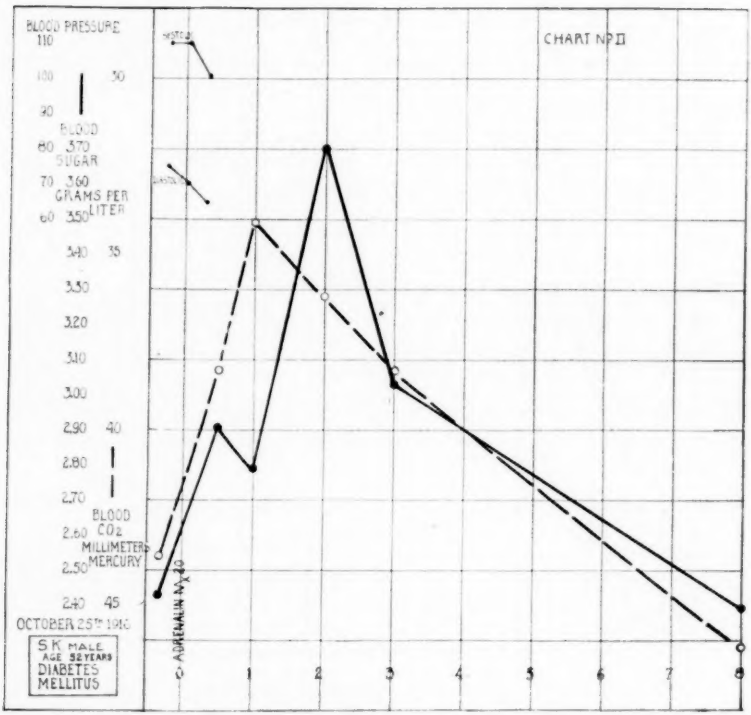


Case 1.—M. M. Female. Age 46 years. Diabetes mellitus of 2 to 3 years' duration.

Oct. 18, 1916, after a fast of 39 hours, adrenalin mg12 injected subcutaneously at 9.30 a.m. Within a few minutes of its administration the patient complained of throbbing in head and throughout body; slight headache. Fibrillary twitching in muscles of arms and trunk; slight hyperpnea. Within 15 minutes after the administration of the drug all subjective symptoms had disappeared.

Pulse increased from 88 to 122, 7 minutes after adrenalin, and then dropped to 90.

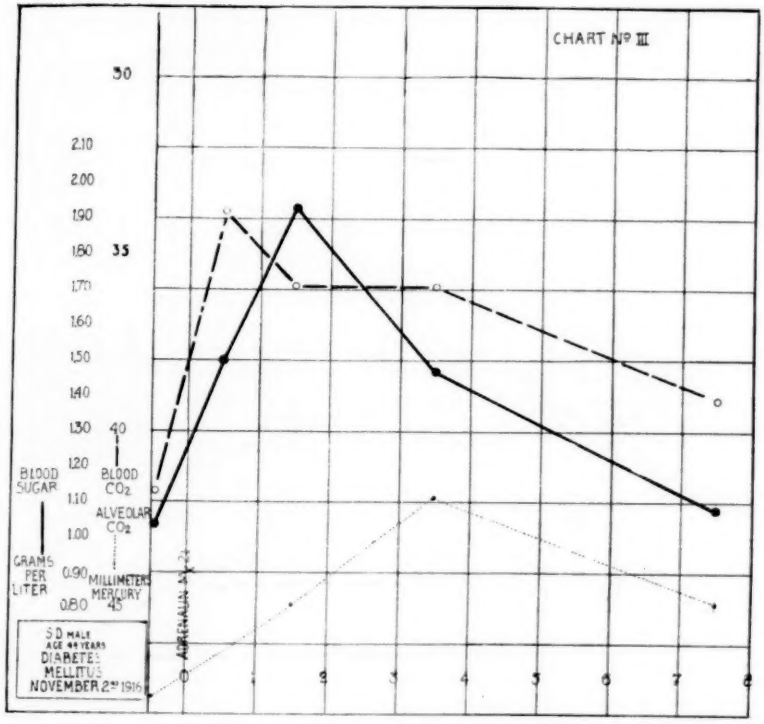
Urine.—Before adrenalin: sugar 0. After adrenalin: sugar 0.



Case 2.—S. K. Male. Age 52 years. Diabetes mellitus of 10 years' duration, and pulmonary tuberculosis of at least 2 years' duration.

Oct. 25, 1916, after a fast of 41 hours, adrenalin mp20 given subcutaneously at 11.30 a.m. There were no subjective or objective symptoms observed. No increase in pulse rate.

Urine.—Before adrenalin: sugar 0. After adrenalin: taken hourly for 4 hours, contained 2.35 gm. of sugar. After this became sugar-free.

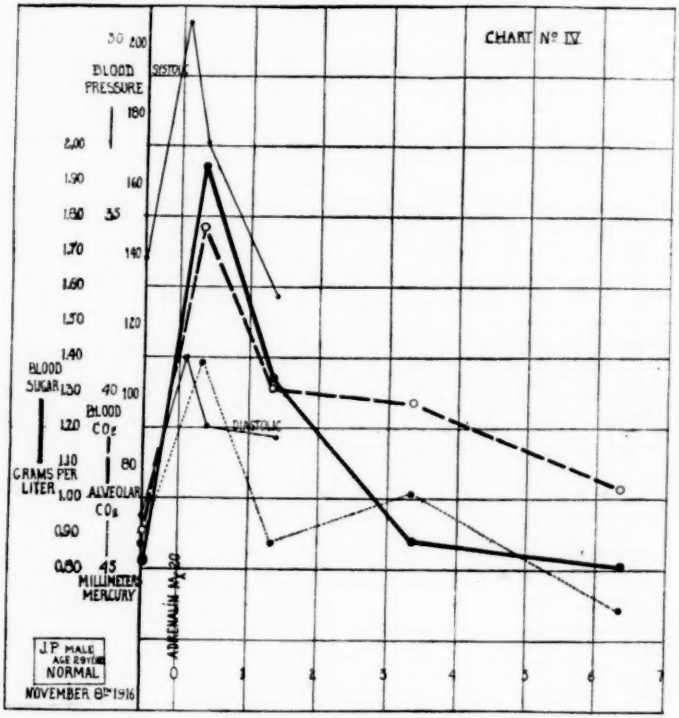


Case 3.—S. D. Male. Age 44 years. Diabetes mellitus of 5 years' duration.

Nov. 2, 1916, after a fast of 23 hours, adrenalin ♏21 injected subcutaneously at 9 a.m. Almost immediately (within 5 minutes) the patient complained of throbbing throughout body, and felt as though "blood was all in legs and stomach." Hyperpnea quite marked. Complete subsidence of subjective symptoms within 10 minutes.

Pulse increased from 78 to 110, 5 minutes after adrenalin was given, and then dropped to 80 15 minutes after the adrenalin had been given.

Urine.—Before adrenalin: sugar 0. After adrenalin: 1st hour, sugar 0; 2nd hour, sugar faint trace; for remainder of the day, sugar 0.

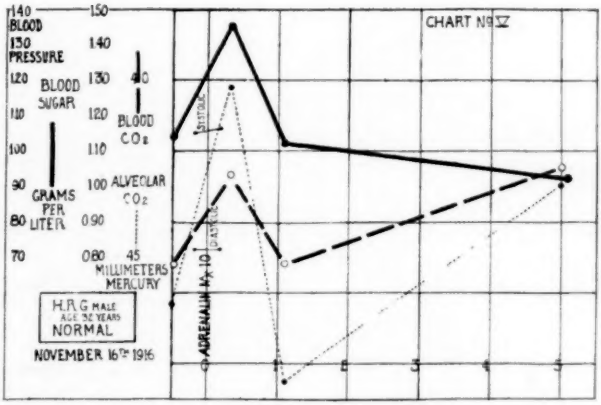


Case 4.—J. P. Male. Age 29 years. Normal.

Nov. 8, 1916, after a fast of 14 hours, adrenalin mg20 injected subcutaneously at 9.40 a.m. Within 3 minutes the patient became very pale and hyperpneic, with palpitation sensations and alternate numbness and tingling in hands and feet. Face showed anxiety and distress although he insisted that he enjoyed the sensations. 15 minutes later all symptoms had disappeared.

Pulse increased from 80 to 115, 5 minutes after adrenalin was given, then dropped to 90.

Urine.—Before adrenalin: sugar 0. After adrenalin: 1st hour, sugar trace; for subsequent 12 hours, sugar 0.



Case 5.—H. R. G. Male. Age 32 years. Normal.

Nov. 16, 1916, after fasting for 11 hours, adrenalin mg10 given subcutaneously at 9.20 a.m. Within 5 minutes mild sensations of constriction of the head, which passed almost immediately. No hyperpnea.

Pulse rate not increased.

Urine.—Contained no sugar at any time.